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NATIONAL RESEARCH COUNCIL OF CANADA
NRC ASSOCIATE COMMITTEE ON SCIENTIFIC CRITERIA FOR
ENVIRONMENTAL QUALITY

TFM AND BAYER 73
LAMPRICIDES
IN THE AQUATIC ENVIRONMENT

PANEL ON TFM AND BAYER 73

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PANEL ON TFM AND BAYER 73
LAMPRICIDES IN THE AQUATIC ENVIRONMENT

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FOREWORD

TFM (3-trifluoromethyl-4-nitrophenol) is the principal lampricide currently licensed for control of the sea lamprey (Petromyzon marinus) in the Great Lakes of North America. A second approved lampricide, Bayer 73 or clonitralid (the 2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide), is also used to a limited extent. Between 1958 and 1983, approximately 1 265 000 kg of TFM and 8400 kg of Bayer 73 were applied to the tributaries of the Great Lakes to control sea lamprey larvae.

Sea lampreys are considered to have played a significant role in the severe reduction of fish populations in the Great Lakes. Although lampricides have been successful in significantly reducing the abundance of sea lampreys, concern has been raised over the possible effects of the repetitive use of lampricides on nontarget organisms in the aquatic environment. In order to address the scientific aspects of this concern, the Subcommittee on Water of the National Research Council of Canada's Associate Committee on Scientific Criteria for Environmental Quality requested that a Scientific Panel be assembled to:

- i) gather and critically evaluate the available information on the fate and effects of the lampricides TFM and Bayer 73 in the aquatic environment of the Great Lakes and extract the relevant scientific criteria; and
- ii) identify critical gaps in the scientific information and recommend research to fill them.

In addressing itself to these tasks, the Scientific Panel examined the available information on the effects of sea lamprey predation on fish, the development of lampricides and their current use patterns. Physicochemical properties, methods of analysis, fate in the aquatic environment and effects on sea lampreys and on nontarget organisms were investigated. Available laboratory and field information was examined. The literature base on which this document is founded consists of published journal articles, theses and unpublished material relating to the use of lampricides contained in reports to the Great Lakes Fishery Commission, as well as data provided by the United States Fish and Wildlife Service and the Canadian Department of Fisheries and Oceans.

The Scientific Panel wishes to make it clear that this document does not evaluate the program for sea lamprey control nor its administration, neither does it examine the socio-economic impact of the use or nonuse of a particular control option. For information of this nature, the reader is referred to the audit of the sea lamprey control and research program available from the Great Lakes Fishery Commission (Chamut 1980).

In its attempt to evaluate information on the possible effects of TFM and Bayer 73 on receptors in the aquatic environment, the Scientific Panel reviewed the scientific literature available prior to February 1985. Although they did not examine the sea lamprey control program per se, the Scientific Panel has considered information on use patterns, treatment techniques, field observations, etc., in an attempt to gain a better insight into ecosystem responses to the use of these chemicals in the aquatic environment.

The following document is composed of two major sections. In the first section, the Scientific Panel summarizes and evaluates the available scientific criteria for TFM and Bayer 73 and recommends research to fill specific gaps in knowledge. The second section contains the detailed information on which the evaluations and recommendations are based.

SUMMARY OF SCIENTIFIC CRITERIA AND RECOMMENDATIONS FOR RESEARCH

1. SUMMARY

After construction of the Welland Canal in 1829, sea lampreys gained access to the entire Great Lakes system. The most pronounced effects of sea lampreys were noted in the upper Great Lakes where they were thought to have contributed largely to the demise of the lake trout (*Salvelinus namaycush*) as well as of other fish species. In response to the decline of the lake trout fishery, research was begun to determine ways of reducing the sea lamprey population. It was known that the life history of the sea lamprey consisted of two major stages: a nonparasitic, stream-dwelling, larval phase and a parasitic, lake-inhabiting phase. Initially, barriers were constructed in a number of tributaries of the Great Lakes to prevent adults from ascending and spawning in the streams. However, the barriers proved to be largely unsuccessful for control. The development of a selective lampricide was begun while the barrier systems were still in use, and culminated in the discovery of TFM (3-trifluoromethyl-4-nitrophenol). The use of selective lampricides was considered appropriate since sea lamprey larvae generally spend four to seven years in the soft bottoms of streams before metamorphosing and descending into the lakes, where they feed on fish. Hence, several year-classes of sea lampreys could be eliminated during a single stream treatment. TFM was successfully applied to several tributaries of Lake Superior in 1958, and its use was expanded to Lake Michigan and Lake Huron in 1960. The sea lamprey population declined substantially while fish populations showed improved growth and survival. Because lamprey predation was thought to have contributed to the failure of a salmonid stocking program in Lake Ontario, TFM treatments were initiated in 1971 in Ontario and in 1972 in New York State. Since sea lampreys had not become numerous in Lake Erie until recently, no lampricide treatments have yet been carried out.

The screening of chemical control agents continued and in 1963 Bayer 73 (clonitralid), the 2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide (Bayer 2353), was found to be highly toxic to the sea lamprey. Because Bayer 73 is also toxic to certain nontarget organisms, it is used as an additive in TFM formulations or used in a granular formulation, to treat slow-moving waters where TFM is largely ineffective.

CHEMISTRY OF TFM AND BAYER 73 (see Section 2.0)

TFM is a halogenated mononitrophenol with the molecular formula $C_7H_5F_3NO$, and a molecular weight of 207.11. The free phenol is very soluble in a number of organic solvents including N,N-dimethylformamide (DMF). The sodium salt of TFM has a solubility in water of 5 g.L^{-1} at 25°C . Aqueous solutions of TFM have a pK_a of

approximately 6. In aqueous solutions, the free phenol of TFM absorbs light at 290 nm, whereas the absorption maximum of the phenolate ion occurs at 394 nm. In field treatments, formulated grade TFM containing approximately 35% active ingredient is used; the remaining 65% of the formulation consists primarily of the carrier DMF and water.

Bayer 73 is a pale yellow powder with a molecular weight of 388.21. Bayer 73 has a solubility in water of $230 \pm 50 \text{ mg.L}^{-1}$ at 25°C, but at pH values below 7, it becomes insoluble. Bayer 73 absorbs light at 330 nm. A 70% active ingredient, wettable powder formulation of Bayer 73 is used in mixtures with TFM. Such a mixture contains 0.5-2% Bayer 73 by weight, although in laboratory studies the ratio of TFM:Bayer 73 is usually 98:2. When applied alone, Bayer 73 is used as a 5% active ingredient granular formulation.

It should be noted that all concentration data reported in this document are based on the weight of active ingredient.

USE PATTERNS (see Section 3.0)

To gain some insight into the effects of TFM and Bayer 73 on both target and nontarget organisms, the Scientific Panel considered it necessary to examine information on use patterns and treatment techniques as they apply to actual field operations.

Of the 5743 streams flowing into the Great Lakes, 433 have been shown to contain sea lampreys. Because of the long larval life-span (typically 4 to 7 years), only a limited number of these streams is treated each year. The stream treatment season usually lasts from early May to late October.

The total amounts of lampricides used to treat lamprey-infested tributaries in Canada and the United States between 1958 and 1983 are listed in the following table:

Lake	Period covered (years inclusive)	Number of streams treated	Number of treatments	Amount of TFM applied (kg)	Amount of powdered Bayer 73 applied (kg)	Amount of granular Bayer 73 applied (kg)
L. Superior	1958-1983	123	672	436 695	3011	1113
L. Michigan	1960-1983	116	442	477 921	1324	391
L. Huron	1960-1983	99	376	286 504	1412	524
L. Ontario	1971-1983	48	166	63 519	257	324
L. Erie	1958-1983	-	-	-	-	-
TOTAL	1958-1983	386	1656	1 264 639	6004	2352^a

^a Excluding the amounts of granular Bayer 73 used in surveys in Canada.

Prior to stream treatments, surveys are conducted to ascertain the presence, distribution and length (age) of resident sea lamprey larvae. If treatment is required, the choice must be made between TFM and the significantly less expensive mixture of TFM and Bayer 73. In reaching a decision, the potential savings are weighed against the increased risk to nontarget organisms and the added complexity of treatment. The mixture is usually employed in large rivers where large quantities of TFM would be required and where the occurrence of highly sensitive nontarget fish species does not present an unacceptable risk. In Canada, in slow-moving waters where TFM is not effective, the 5% granular formulation of Bayer 73 is applied as a bottom toxicant.

Water chemistry is known to affect lampricide toxicity (see Section 7.0), so 24-hour bioassays are usually conducted in stream water immediately prior to treatment to determine the range of concentrations of lampricide to be applied. The concentrations of TFM used in the Great Lakes basin range from 1 to 17 mg.L⁻¹. In a treatment, lampricide is metered into the stream over 8 to 20 hours, depending on the length and complexity of the tributary system to be treated. Concentrations of TFM throughout the stream are monitored every 1 to 2 hours and are adjusted as required, to maintain a specific concentration throughout the time of treatment. In the United States, granular Bayer 73 is applied at a rate of 5.6 to 8.4 kg.ha⁻¹, while in Canada the maximum rate of application is 11.2 kg.ha⁻¹. These rates are predicted to yield concentrations in the bottom 5 cm of water between 11.5 and 17.2 mg.L⁻¹ (U.S.A.) and 23 mg.L⁻¹ (Canada). However, these levels are unlikely to be reached because less than 40% of the active ingredient is released over a 24-hour period.

Following treatment, sea lamprey larvae may not become reestablished for several years, particularly in small streams. Stream surveys are conducted to determine whether recolonization has occurred and hence, the need for retreatment.

METHODS OF ANALYSIS FOR TFM AND BAYER 73 (see Section 4.0)

The methods used for measuring the concentrations of TFM and Bayer 73 in stream water during a treatment differ between the United States and Canada. In the United States, TFM and Bayer 73 levels are determined by high performance liquid chromatography (HPLC) and gas-liquid chromatography (GLC), respectively. Current methodology is moving towards a system in which both lampricides are measured simultaneously by HPLC. The sensitivity is < 0.005 mg.L⁻¹. In Canada, TFM is monitored using a colorimetric technique with an analytical accuracy of 0.1 mg.L⁻¹. Concentrations of Bayer 73 are not measured.

TFM residues in tissues are analyzed using a GLC method with a sensitivity of 0.01 µg.g⁻¹ wet weight. Residues of Bayer 73 in tissues and sediments are determined using a GLC technique with a sensitivity of 0.01 µg.g⁻¹ wet weight.

TFM AND BAYER 73:
LAMPRICIDES IN THE AQUATIC ENVIRONMENT

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FATE OF TFM AND BAYER 73 IN THE AQUATIC ENVIRONMENT (see Section 5.0)

Abiotic Processes (see Section 5.1)

TFM does not undergo volatilization or hydrolysis, but it is photodegraded in stream water, yielding several photoproducts. It has been predicted that the photolytic half-life of TFM in streams would be 3.5 days in spring, 3 days in summer and 5 days in autumn, assuming sunny weather and a stream depth of 55 cm. In ponds with an average depth of 1.25 m, the observed half-life was 9 days.

Two of the photoproducts of TFM have been identified as 2,5-dihydroxybenzoic acid and 2-(3-trifluoromethyl-4-nitrophenoxy)-5-hydroxybenzoic acid. Five other photoproducts have also been tentatively identified. These photoproducts have been detected downstream from TFM treatment sites.

No information is available concerning the volatilization of Bayer 73 from aqueous media. Bayer 73 does not hydrolyze in natural surface waters. The photolysis of Bayer 73 in distilled water has been studied in the laboratory. The removal of 95% of ^{14}C -Bayer 2353 required 14 days in distilled water illuminated with UV (290-405 nm) light.

The abiotic binding of TFM, RTFM (3-trifluoromethyl-4-amino-phenol) and Bayer 73 to different sediment types was evaluated in laboratory experiments using 20 mL of 1 mg.L⁻¹ solutions of radiolabelled materials and 2 g (dry weight) of sediment. The solutions and sediments were allowed to equilibrate (> 16 h) on an orbital shaker. Adsorption isotherms indicated that the adsorption maxima were not exceeded. The adsorption of TFM progressively increased as the sediment type was changed from sand to sand/silt mixtures to silt. At pH 7, the adsorption of TFM was 0.3 $\mu\text{g.g}^{-1}$ on sand compared with 3.5 $\mu\text{g.g}^{-1}$ on silt. In addition, adsorption increased as the pH declined for each sediment type. The amount of TFM adsorbed to silt increased from 1.1 $\mu\text{g.g}^{-1}$ at pH 9 to 5.8 $\mu\text{g.g}^{-1}$ at pH 6.

In desorption experiments, the amount of TFM released over 24 hours was dependent on the sediment type and temperature. At 20°C, there was a 100% release of TFM from sand compared with a 65% release from silt. At 5°C, the desorption from sand was 50% while the release from silt declined to 35%.

RTFM was consistently adsorbed to a greater extent than TFM, regardless of pH or sediment type. For silt, the quantity of RTFM adsorbed fluctuated between 5.5 and 7.0 $\mu\text{g.g}^{-1}$ over the pH range 6 to 9, whereas for TFM, the adsorption progressively decreased from 5.8 to 1.1 $\mu\text{g.g}^{-1}$. RTFM was adsorbed strongly to all sediments; less than 20% of that adsorbed was released at pH 7 in 24 hours.

The adsorption of Bayer 73 to sediment was affected largely by the sediment type. At pH 7, the adsorption was greatest on silt ($9.3 \mu\text{g.g}^{-1}$), intermediate on sand/silt mixtures ($8.8 \mu\text{g.g}^{-1}$) and least on sand ($5.0 \mu\text{g.g}^{-1}$). Bayer 73 was adsorbed to a greater extent than either TFM or RTFM on all sediment types.

The effect of pH on adsorption of Bayer 73 varied, depending on the type of sediment. The pH appeared to have no effect on adsorption on silt, because the levels fluctuated from 2.8 to $4.0 \mu\text{g.g}^{-1}$ over the pH range 6.5 to 9.0. However, adsorption on sand showed a distinct decline with increasing pH, from $5.4 \mu\text{g.g}^{-1}$ at pH 6.5 to $0.9 \mu\text{g.g}^{-1}$ at pH 9. Below pH 6.5, Bayer 73 precipitates from solution.

The desorption of Bayer 73 was related to sediment type but was independent of temperature. Using a technique of frequent water replacement over 24 hours, the release from silt, sand and sand/silt mixtures as a percentage of the initial amount adsorbed, was 65%, 25% and 15% respectively.

Biotic Processes (see Section 5.2)

The potential transformation of TFM and Bayer 73 by micro-organisms was investigated in aerobic and anaerobic environments in the laboratory using water and sediment and sediment-free systems. TFM remained unaltered under aerobic conditions for periods up to 80 days, indicating an absence of microbial action. In anaerobic environments, TFM was microbially reduced to RTFM in 5 to 20 days, but was not further degraded.

In contrast, Bayer 2353 was subject to microbial degradation. Radiolabelled Bayer 2353 was added to water at a concentration of 1 mg.L^{-1} and incubated with river sediments under static conditions for 128 days at 25°C . The half-lives in the water and river sediments were 0.83 and 3.9 days respectively. The major transformation product was 2',5-dichloro-4'-aminosalicylanilide.

Biological Cycling (see Section 5.2.2)

The pharmacokinetics of TFM in aquatic macrophytes, algae and benthic invertebrates have been studied in model stream systems (initial TFM concentration 9 mg.L^{-1} , 24-hour exposure, static conditions). TFM was rapidly taken up, but reached only low levels in tissue, before being rapidly eliminated. Although the reported bioconcentration factors (BCFs) may be low because of the experimental design, they were all less than 100, indicating a relatively low potential for bioconcentration. Soft-bodied organisms (e.g. annelids, $\text{BCF} = 50$) tended to accumulate more TFM than did hard-bodied organisms (e.g. crayfish, $\text{BCF} = 1$). After transfer to TFM-free water, all of the

organisms studied, except annelids, eliminated most of their body burdens within 24 hours. In annelids, half-lives of approximately 7 months were predicted. These laboratory findings were confirmed in the field where a stream was treated with 11 mg.L^{-1} of TFM for 12 hours. In the 96-hour period following treatment all species examined, with the exception of annelids, had cleared 93-99% of the TFM initially concentrated. No annelids were found after the treatment, indicating that they may have been eliminated. Although few studies of TFM metabolism in invertebrates have been carried out, it has been demonstrated that the aquatic midge (*Chironomus tentans*) has the ability to biotransform TFM. For example, after a 12-hour exposure to 0.224 mg.L^{-1} of ^{14}C -TFM, followed by a 16-hour recovery period, roughly 45% of the TFM had been metabolized.

The concentrations of Bayer 2353 in aquatic invertebrates that had been exposed to approximately 0.001 mg.L^{-1} of ^{14}C -Bayer 2353 for 24 hours, had reached equilibrium and corresponded to BCFs of less than 100. As with TFM, the uptake of Bayer 2353 appeared to be greater in soft-bodied organisms. Rapid clearance occurred when exposure ceased. The aquatic midge was able to eliminate 90% of its accumulated residues within 48 hours of transfer to clean water. This ability was thought to be related to its biotransformation capability. Two metabolites were found: one was identified as ^{14}C -chlorosalicyclic acid while the other was the conjugated form of either Bayer 2353 or chlorosalicyclic acid.

Both laboratory and field studies using various species of nontarget teleost fish have demonstrated that TFM and Bayer 73 are rapidly taken up and conjugated with glucuronic acid, primarily in the liver and kidneys, and then undergo biliary and renal excretion. TFM and Bayer 73 levels in the blood, brain and muscle tissues remain low while those in the liver and kidneys are higher but still less than those in the bile. For example, concentrations in the blood and brain of largemouth bass exposed to 1 mg.L^{-1} of ^{14}C -TFM for 12 hours were less than $2 \text{ } \mu\text{g.g}^{-1}$. In the same experiment, the concentrations in the kidney, liver and bile were 15.35, 22.52 and $696.4 \text{ } \mu\text{g.g}^{-1}$ respectively. In general, TFM and Bayer 73 are cleared from all tissues within 24 hours after transfer of the fish to clean water. The bile continues to accumulate TFM and Bayer 73 after the exposure has ended, probably as a result of residue pools emptying into it. For instance, in largemouth bass that had been exposed to 1 mg.L^{-1} of TFM for 12 hours, the concentrations of TFM in the kidney, liver, brain and blood had declined to $2.35 \text{ } \mu\text{g.g}^{-1}$ after 24 hours in TFM-free water. Concentrations of TFM in the bile increased from $823.6 \text{ } \mu\text{g.g}^{-1}$ at the end of the exposure period to $1263.1 \text{ } \mu\text{g.g}^{-1}$ 4 hours later, before falling to $796.3 \text{ } \mu\text{g.g}^{-1}$ at 24 hours postexposure.

In contrast to teleosts, TFM is taken up by sea lamprey larvae much more rapidly and to a greater extent. In addition, the degree of conjugation is much less. For example, during a 4-hour exposure to 0.25 mg.L^{-1} of ^{14}C -TFM, lamprey larvae accumulated approximately $6 \text{ } \mu\text{g.g}^{-1}$, of

which only 7% was conjugated. Under the same exposure conditions, rainbow trout accumulated only $2.5 \mu\text{g.g}^{-1}$ and 70% was conjugated. No information is available on the environmental fate of the TFM contained in dead or moribund lampreys. Field observations have shown that moribund larvae are consumed by sea gulls. No information on the pharmacokinetics of Bayer 73 in sea lampreys is available.

Although field observations have not identified any problem, no laboratory studies are available concerning the uptake, distribution or elimination of TFM and Bayer 73 by amphibians, reptiles, aquatic birds or aquatic mammals. A laboratory study using rats has shown that 60% of an injected dose of 12 mg.kg^{-1} TFM was excreted in the urine within 24 hours, 6% in the form of unaltered TFM. Another laboratory study on the metabolism of ^{14}C -Bayer 2353 by rats showed that about 50% of the dose administered was excreted in the urine, mainly in the form of 2',5-dichloro-4'-aminosalicylanilide.

MODE OF ACTION OF TFM AND BAYER 73 (see Section 6.0)

The mode of action of TFM in sea lampreys and nontarget organisms remains largely undefined. Early observations on moribund lampreys suggested that death occurred because of respiratory and circulatory collapse. The results of a recent study indicate that the adverse effects of TFM on ion-uptake cells of the gills may be a contributing factor in the death of sea lampreys.

At the biochemical level, there is some evidence that TFM acts by uncoupling oxidative phosphorylation in bacteria, aquatic invertebrates, sea lampreys and rats. This may also occur in fish although experimental evidence is lacking. In addition, fish exhibit excessive mucus production by the gills and display classic symptoms of anoxia.

The mode of action of Bayer 73 in sea lampreys and nontarget organisms is unknown. However, it appears to act in a manner similar to that of TFM: as a possible uncoupler of oxidative phosphorylation in sea lampreys and invertebrates.

Although the mode of action of TFM is not definitely known, its selective toxicity to sea lampreys relative to teleosts is thought to be due to two factors. First, sea lampreys take up more TFM than do rainbow trout under identical exposure conditions. Second, the ability to biotransform and detoxify TFM via a conjugation reaction with glucuronic acid appears to be absent or minimal in sea lampreys, in contrast to its active presence in teleosts.

ENVIRONMENTAL FACTORS AFFECTING TOXICITY (see Section 7.0)

Information on how environmental factors affect the toxicities of TFM, Bayer 73 and the 98:2 mixture to various aquatic organisms is available from 96-h LC_{50} tests. Variations in such factors as water temperature, hardness, pH and dissolved oxygen must be taken into account in observations of effects in natural aquatic ecosystems.

Based on laboratory studies, water temperature in the range of 7 to 17°C has little effect on the acute toxicities of TFM, Bayer 73 and the 98:2 mixture to lampreys and nontarget fish. However, observations from the field have indicated that the efficacy of TFM and Bayer 73 is reduced at water temperatures less than 4°C. Increasing water temperature has a slight effect on the toxicity of TFM to coldwater fish; for example, the 96-h LC_{50} for rainbow trout declined from 2.13 to 1.74 $mg.L^{-1}$ as the water temperature increased from 7 to 17°C (water hardness 44 $mg.L^{-1}$ $CaCO_3$, pH 7.5).

The toxicity of TFM to mayfly nymphs was unaffected by temperature. No data are available concerning the effect of temperature on the toxicity of the 98:2 mixture or Bayer 73 to invertebrates.

At pH values of 6.5 to 7.5, changes in water hardness had no significant effect on the toxicity of TFM to lampreys. In contrast, at higher pHs, increasing the water hardness decreased the toxicity to nontarget fish. At pH 8.1, the 96-h LC_{50} for rainbow trout increased from 9.0 to 17.3 $mg.L^{-1}$ when water hardness was increased from 10 to 300 $mg.L^{-1}$ $CaCO_3$. For invertebrates, increases in water hardness also decreased acute toxicity. It is postulated that as water hardness increases, the phenolate ion is complexed, thus making the TFM less available for uptake. Changes in water hardness had little or no effect on the toxicity of Bayer 73 or the 98:2 mixture.

Of the environmental parameters examined, pH has the greatest influence on acute toxicity of TFM to lampreys, nontarget fish and invertebrates. For example, the 96-h LC_{50} of TFM for lampreys increased from 0.172 to 1.30 $mg.L^{-1}$ as the pH increased from 6.5 to 8.5 at a water hardness of 44 $mg.L^{-1}$ $CaCO_3$ and temperature of 12°C. Similarly, for rainbow trout, the 96-h LC_{50} increased from 1.0 to 5.4 $mg.L^{-1}$ over the same pH range. Decreased toxicity at higher pH values is believed to be attributable to a lower uptake. At pH values above 6.07 (the pK_a of TFM), the concentration of lipid-soluble free phenol decreases while that of the phenolate ion increases.

Similar effects of changes in pH on the acute toxicity of Bayer 73 have been recorded, although the change in toxicity is not as pronounced. In addition, at pH values below 6.5, Bayer 73 precipitates.

Reduced levels of dissolved oxygen may stress fish, making them more susceptible to lampricides. Field observation data based on

the 98:2 mixture, have indicated the occurrence of fish kills during stream treatments where dissolved oxygen levels were 0.6 to 2.4 mg.L⁻¹. Dissolved oxygen levels below 4 mg.L⁻¹ are considered stressful for most fish species (Doudoroff and Shumway 1970; NRCC 1975).

EFFECTS ON LAMPREYS (see Section 8.0)

In general, lamprey species that are native to the Great Lakes are more resistant to the acute action of TFM than sea lampreys. For example, 9-h LC₅₀ values for the sea lamprey (*Petromyzon marinus*) and native genera (*Ichthyomyzon* and *Lampetra*) were found to be 1.4, 2.0 and 2.5 mg.L⁻¹ TFM, respectively, at 7°C and total alkalinity of 45 mg.L⁻¹ CaCO₃. No information concerning the differential toxicity of Bayer 73 or the TFM:Bayer 73 mixture is available. Although native lampreys tend to be more resistant than sea lampreys, the difference in toxic response is not sufficient to selectively remove only sea lampreys during typical treatments and, hence, a decrease in the population of native lampreys has been observed in many of the treated streams.

Estimates of populations of sea lampreys in the Great Lakes have declined by approximately 90% since their peak abundance in 1961. Although little information is available on population effects of Bayer 73, in one study where Bayer 73 was applied to a slow moving water body, the sea lamprey ammocoete population declined by 89%. Posttreatment assessments have confirmed this finding.

Eggs and embryos are more resistant to TFM than ammocoetes; for example, the 24-h LC₅₀ declined from 3.57 mg.L⁻¹ at the hatching stage to 0.56 mg.L⁻¹ at the 7-cm larval stage. Exposure to TFM during embryonic development has been found to increase the frequency of abnormalities, which often results in later mortality.

Burrowed larvae appear more resistant than free-swimming larvae to TFM, Bayer 73 and the 98:2 mixture. The 12-h LC₅₀ value increased from 3.0 to 5.39 mg.L⁻¹ TFM and from 0.092 to 0.280 mg.L⁻¹ Bayer 73 for free swimming and burrowed ammocoetes, respectively. Increased mortality in free-swimming larvae is believed to be related to greater uptake due to increased activity, reduced protection or greater stress in the absence of a substrate.

Since the introduction of lampricide treatments, the average size of ammocoetes, recently metamorphosed individuals and adults has increased. In addition, especially in the upper Great Lakes, there has been a tendency for a shift in the sex composition of the sea lamprey population towards fewer males. For example, in Lake Superior, the proportion of males has decreased from 70% in 1960-62 to 30-40% in 1978.

Usually in the year following treatment, there has been a dramatic decline in the number of adult lampreys entering treated

streams. Although the mechanisms for this response have yet to be elucidated, it has been suggested that adults appear to prefer streams already populated by ammocoetes.

EFFECTS ON AQUATIC MACROPHYTES AND ALGAE (see Section 9.0)

Considering the concentrations and exposure times used in sea lamprey control operations, TFM does not appear to have adverse effects on aquatic macrophytes or algae, with the possible exception of a temporary reduction in their rate of growth. In experiments using a recirculating stream system, it has been shown that for some aquatic macrophytes exposed to 6-7 mg.L⁻¹ TFM for 10 hours, a slight reduction in growth rate occurred in the 2-week period following treatment. Reductions in growth rate of 5-10% for Canadian pondweed (Elodea canadensis) and approximately 20% for European milfoil (Myriophyllum spicatum) were observed.

Most species of algae exhibit an algistatic response when exposed to TFM. With the exception of Chlorella pyrenoidosa (which was more resistant), exposed species suffered a 50% reduction in growth at TFM concentrations of < 10 mg.L⁻¹ for 96 hours. In field studies using an experimental stream and an exposure of 9 mg.L⁻¹ TFM for 24 hours, no effect was observed on the relative occurrence of different species or on the biomass of the standing crop. The effects of Bayer 73 or the 98:2 mixture on aquatic plants and algae are not known.

EFFECTS ON INVERTEBRATES (see Section 10.0)

The sensitivities of stream invertebrates to TFM, the 98:2 mixture and Bayer 73 differ significantly among and within groups. Although most of the data on acute toxicity have been derived from the laboratory, field studies and field observations have also contributed useful information regarding the toxicological effects of lampricides on invertebrates.

Based on acute lethality tests, aquatic annelids are very sensitive to TFM. The 24-h LC₅₀ for Tubifex tubifex in soft water (hardness = 20 mg.L⁻¹ CaCO₃) was 3.43 mg.L⁻¹. In contrast, crustaceans were relatively unaffected by TFM. Considerable variability in sensitivity to TFM was observed among aquatic insects. In the Diptera, blackfly larvae (Simulium pugetense) were sensitive, having a 24-h LC₅₀ of 6.1 mg.L⁻¹ in water of hardness 211 mg.L⁻¹ CaCO₃, pH 7.79 and temperature 12°C. Among the Ephemeroptera (mayflies), Baetis sp., Hexagenia sp., and Cloeon sp. were sensitive, with 24-h LC₅₀s of 6.9, 7.9 and 11.1 mg.L⁻¹ of TFM respectively. In soft water at 17°C, the 24-h LC₅₀ for Hexagenia sp. nymphs was 1.5 mg.L⁻¹. The sensitivity of caddisflies (Trichoptera) was also variable. Chimarra obscura was the most sensitive organism tested (24-h LC₅₀ of 3.8 mg.L⁻¹), while

Limnephilus consocius was the most resistant (24-h LC_{50} > 39.0 mg.L⁻¹). Stoneflies (Plecoptera) were all relatively resistant to TFM (24-h LC_{50} s ranged from 16.7 to >34.0 mg.L⁻¹). Dobsonflies (Megaloptera) and dragonflies (Odonata) were very resistant to TFM, having 24-h LC_{50} s of >26.0 and >38.0 mg.L⁻¹, respectively. Pelycypod molluscs showed an intermediate sensitivity, with 24-h LC_{50} values ranging from >11.2 to >18.0 mg.L⁻¹.

From field observations, the most apparent effect of a TFM treatment is an immediate reduction in macroinvertebrate density. It has been suggested that increased downstream drift, together with mortalities of sensitive organisms, is responsible for this decline. However, this effect appears to be temporary since various investigators have found recolonization as early as 4 weeks after TFM treatment.

Similar trends in acute toxicity were found for the 98:2 mixture. Turbellarians, oligochaetes and leeches were very sensitive; their 24-h LC_{50} s ranged from 1.5 to 5.2 mg.L⁻¹ in water of hardness 100-136 mg.L⁻¹ CaCO₃, pH 8.2-8.3 and temperature 12°C. Among crustaceans, daphnids were most sensitive (24-h LC_{50} of 7.35 mg.L⁻¹). Among aquatic insects, there was great variability in sensitivity. Within the Diptera, Simulium sp. (blackfly larvae) had a 24-h LC_{50} of 3.45 mg.L⁻¹ whereas Atherix sp. (snipefly larvae) had a 24-h LC_{50} > 100 mg.L⁻¹. Among Ephemeroptera, Hexagenia sp. was the most sensitive species, with a 24-h LC_{50} of 4.0 mg.L⁻¹. The Plecoptera were resistant; Paragnetina sp. had a 24-h LC_{50} of 15.4 mg.L⁻¹. Odonata and Megaloptera were unaffected by the mixture (24-h LC_{50} s > 100.0 mg.L⁻¹). It should be pointed out that levels of Bayer 73 in stream treatments using the 98:2 mixture rarely reach lethal concentrations.

Bayer 73 is much more toxic to many aquatic invertebrates than either TFM or the 98:2 mixture. Among the most sensitive organisms are turbellarians, oligochaetes and leeches, which all have 24-h LC_{50} s < 0.50 mg.L⁻¹. Of the crustaceans, the cladoceran, Daphnia pulex, was very sensitive (24-h LC_{50} of 0.8 mg.L⁻¹). Among Diptera, the blackfly, Simulium sp., and the midge, Chironomus tentans, were very susceptible (24-h LC_{50} s of 0.255 and 0.57 mg.L⁻¹, respectively). In contrast to its sensitivity to TFM and the 98:2 mixture, Hexagenia sp. was moderately resistant to Bayer 73 (24-h LC_{50} of 6.9 mg.L⁻¹). Caddisflies were relatively sensitive (24-h LC_{50} s ≤ 2.45 mg.L⁻¹). All molluscs tested were found to be very sensitive to Bayer 73, having 24-h LC_{50} s less than 0.4 mg.L⁻¹.

Based on the available information, it appears that the use of TFM will adversely affect populations of Turbellaria, Oligochaeta and some Arthropoda. Among arthropods, this includes certain species of mayflies (Hexagenia sp., Baetis sp. and Cloeon sp.), caddisflies (Chimarra sp.) and blackflies (Simulium sp.). In addition, some organisms, such as the mayfly, Baetis sp., the caddisfly, Dolophilodes sp., and the amphipod, Gammarus sp., become more active in response to

TFM and tend to drift. Their fate has not been established. The depletion in fauna sometimes observed after treatments is probably the combined result of the direct toxic effects of TFM, stimulation of the rate of drift and predation. The observed depletions of stream biota during and immediately after treatment with TFM are usually transitory. Potential mechanisms for recolonization include drift from untreated portions of the watershed, movement from the hyporheos, upstream migration and oviposition by flying insects. However, these mechanisms have not been adequately documented and do not apply to all species. Most studies conducted to date have not been at the species level and have not considered preferred habitats, species successions and/or life cycles. The recovery of communities is a complex process. Species with univoltine life cycles, for example, which are present as populations of uniform age at the time of treatment, cannot "recover" in the same way as a species that has a mixed population of adults and juveniles.

Because invertebrate populations have apparently not been eliminated, some mechanisms of recolonization must be available. TFM treatments, which normally occur every 3 or 4 years, have not eliminated even sensitive species, such as Hexagenia sp. (Schuldt 1984). The fact that the sea lamprey control program treats only 20 to 30% of the infested streams in a given year would also reduce the long-term effects on the ecosystem. A more complete understanding of how populations survive and recover from chemical treatments will require species-by-species studies of their individual habitat requirements, life histories, biology and behavior.

It has been suggested that invertebrates which possess hard exoskeletons, or are case builders, are less vulnerable to lampricides than soft-bodied forms. A relationship between permeability and type of body covering remains to be established.

The macroinvertebrates appearing most affected are those that feed primarily on small particles. This suggests that TFM may be bound to particulate matter and released once in the gut, thereby acting as a stomach or enteric poison. Factors such as the binding of TFM to fine particles, the feeding habits of individual species and the effect of gut pH on adsorption-desorption processes of TFM may be useful in explaining the different sensitivities of affected species.

In general, test results for the 98:2 mixture are similar to those for TFM. It appears that molluscs are more susceptible to the mixture than to TFM, and that some populations of snails and clams would be reduced. However, it should be remembered that the mixture is normally used in large rivers where substantial amounts of TFM would otherwise be required.

Bayer 73 is not as selectively toxic to sea lampreys as TFM or the 98:2 mixture. It is more toxic to a larger number of organisms, and its use would likely result in significant declines in turbellarians,

oligochaetes, clams and snails, as well as some midges and caddisflies. Resistant invertebrates include some insect larvae, notably snipeflies, mayflies, dobsonflies and dragonflies, as well as some crustaceans such as amphipods, isopods and crayfish. It should be noted that the use of Bayer 73 is confined to small portions of streams and lentic habitats that represent only a small percentage of the total stream system undergoing treatment. Although a localized impact on some invertebrate species would be expected, the implications for entire aquatic ecosystems would be far less severe.

EFFECTS ON TELEOST FISH (see Section 11.0)

Teleosts are less susceptible to lampricides than sea lamprey larvae. If no other toxicants are present, laboratory and field data for TFM and the 98:2 mixture indicate that the most susceptible species of large fish are suckers (Catostomus spp.), bullheads (Ictalurus spp.) and channel catfish (Ictalurus punctatus). Other fish occasionally affected include northern pike (Esox lucius), walleye (Stizostedion vitreum vitreum) and a number of salmonids. Centrarchids are the most resistant. For example, the 24-h LC_{50} s (temperature 12°C, hardness 44 mg.L⁻¹ CaCO₃, pH 7.4) ranged from 7.05 mg.L⁻¹ TFM for the bluegill sunfish (Lepomis macrochirus) to 1.05 mg.L⁻¹ TFM for channel catfish. Other species were intermediate in sensitivity and had 24-h LC_{50} s ranging from 1.46 to 3.57 mg.L⁻¹ TFM.

Based on qualitative information in field reports, a number of species of forage fish, e.g. common shiner (Notropis cornutus), johnny darter (Etheostoma nigrum) and longnose dace (Rhinichthys cataractae), have occasionally suffered some mortality during treatments. Populations of the stonecat Noturus flavus, a very sensitive species, have been severely reduced. In general, however, fish kills have been minor and localized. No species was observed to have been eradicated from tributaries of the Great Lakes.

Fish in spawning condition are more susceptible to TFM and the 98:2 mixture than are juveniles. There is some controversy concerning the sensitivity of early life stages of teleosts to TFM. All early life stages of walleyes exposed to TFM were more resistant than were sea lamprey larvae. For instance, the 12-h LC_{25} (temperature 12°C, hardness 44 mg.L⁻¹ CaCO₃, pH 7.5) ranged from 1.89 mg.L⁻¹ for 12-h green eggs to 4.70 mg.L⁻¹ for sac fry. The 12-h LC_{50} for sea lamprey larvae was only 1.46 mg.L⁻¹. For rainbow trout, however, there is some evidence of adverse delayed effects. Green eggs exposed to 2.86 mg.L⁻¹ TFM for 24 hours (temperature 10°C, hardness 354 mg.L⁻¹ CaCO₃, pH 7.6) all survived for 7-10 days, and then died.

Bayer 73 is much more toxic to all species of teleost fish than is TFM or the 98:2 mixture. The 24-h LC_{50} s (temperature 12°C, hardness 40-48 mg.L⁻¹ CaCO₃, pH 7.2-7.6) ranged from 0.143 mg.L⁻¹ for

carp (Cyprinus carpio), the most tolerant species, to 0.052 mg.L^{-1} for rainbow trout, the most susceptible species. Because Bayer 73 is used in granular form as a bottom toxicant, most fish can escape its toxic effects. Problems arise when very large areas are treated and bottom-dwelling fish cannot escape. Species that would be affected in such situations include logperch, darters, suckers, bullheads and sculpins.

The newly fertilized eggs of salmonids have been shown to be resistant to Bayer 73. For example, the 24-h LC_{50} s (temperature 12°C , hardness $44 \text{ mg.L}^{-1} \text{ CaCO}_3$) for eggs of coho salmon (Oncorhynchus kisutch), chinook salmon (Oncorhynchus tshawytscha) and lake trout were >4.25 , >2.55 and 0.88 mg.L^{-1} respectively.

Teleosts are sometimes killed in stream treatments, but it is often difficult to determine the causes because of the presence of other toxic substances in the stream. If the toxic burden within organisms is already high, the addition of any other toxicant could induce mortality, even if the applied rate was less than the threshold of toxicity.

The toxicities of TFM and Bayer 73 in separate mixtures with a number of toxicants, including ammonia, carbaryl, cyanide, Delnav, DDT, endrin, malathion, nitrite, tannic acid, toxaphene, cadmium, copper, zinc and chlorine were evaluated on three species of fish: rainbow trout, white suckers (Catostomus commersoni) and fathead minnows (Pimephales promelas). In all cases, the toxicities were additive or less than additive, indicating an absence of synergistic behavior.

EFFECTS ON AMPHIBIANS AND REPTILES (see Section 12.0)

It has been observed that some species of frog larvae are killed during stream treatments with TFM. The 96-h LC_{50} s for larvae of the gray tree frog (Hyla versicolor), leopard frog (Rana pipiens) and bullfrog (Rana catesbeiana) (temperature 17°C , water hardness $44 \text{ mg.L}^{-1} \text{ CaCO}_3$, and pH 6.8-7.0), were 0.78, 1.09 and 1.40 mg.L^{-1} respectively. Turtles exposed to TFM under conditions simulating a stream treatment were not killed. No data are available on the toxicities of the 98:2 mixture or Bayer 73 to either amphibians or reptiles.

EFFECTS ON BIRDS (see Section 13.0)

The acute oral toxicities of TFM, the 98:2 mixture and Bayer 73 have been recorded for a number of bird species. For example, the LD_{50} values for mallard ducks (Anas platyrhynchos) exposed to TFM, the 98:2 mixture and Bayer 73 were 458, 472, and $>2000 \text{ mg.kg}^{-1}$ respectively. The formulated grade TFM was more toxic to mallards ($\text{LD}_{50} = 308 \text{ mg.kg}^{-1}$) than was the analytical grade TFM ($\text{LD}_{50} = 458 \text{ mg.kg}^{-1}$), suggesting an increased absorption of TFM due to the carrier, N,N-dimethylformamide.

No mortalities were recorded for mallard ducks in the 14- to 21-day period following exposure to lampricide-contaminated water for 48 hours. Some birds exposed to 50 mg.L⁻¹ showed signs of lethargy and incoordination; however, all exposed individuals recovered within 24 to 48 hours after exposure.

EFFECTS ON MAMMALS (see Section 14.0)

Information on the effects of TFM, the 98:2 mixture and Bayer 73 to mammals is contained primarily in studies that address the requirements of the United States Environmental Protection Agency for pesticide registration. As such, much of the information does not appear in refereed scientific journals. However, the Scientific Panel considers the material to be useful when attempting to evaluate all available information relating to the toxicological effects of lampricides on both target and nontarget organisms.

No adverse effects were observed on feed consumption, feed efficiency, body weight, clinical parameters or reproductive performance in rats or hamsters fed either TFM or the 98:2 mixture at levels up to 5000 mg.kg⁻¹ TFM and 100 mg.kg⁻¹ Bayer 73 daily for up to two years. In addition, TFM had no carcinogenic, teratogenic or mutagenic effects.

Bayer 73 had no effect on the feed consumption, body weight or clinical parameters of dogs fed up to 1000 mg.kg⁻¹ daily for 180 days. Rats and hamsters receiving up to 5000 mg.kg⁻¹ of Bayer 73 in their daily diet, exhibited a dose-related depression in body weight, although clinical data based on hematology, blood chemistry and urinalysis showed no significant differences from those of the controls. A postmortem histological examination of the rats revealed no pathological changes related to the treatment. In addition, Bayer 73 exhibited no teratogenic, mutagenic or carcinogenic properties.

Based on the available information from these studies, mammals would be unlikely to suffer any toxic effects from either dermal or dietary exposure to lampricide-treated water.



2. RECOMMENDATIONS FOR RESEARCH

Based on the information presented in this document, the Scientific Panel has grouped the recommendations for research into three categories according to the following rationale:

Category A: critical scientific information required to adequately examine specific concerns over the potential adverse effects that the repetitive use of TFM and Bayer 73 could have on nontarget organisms.

Category B: scientific information that would add to the available data base on the use of lampricides and an understanding of observed phenomena. This information would complement the need identified in Category A, but is less critical.

Category C: scientific information that would provide useful knowledge related to lampricide use, but that is not of a pressing need for protection of the environment.

CATEGORY A

1. TFM formulations currently used in field applications consist of approximately 35% active ingredient and 65% other materials, primarily the carrier substance N,N-dimethylformamide (DMF) and water. With the exception of DMF and water, the identity of the other ingredients is unknown. Bayer 73, sometimes used in a mixture with TFM, is made up of 70% active ingredient and 30% other materials. In addition, the fate of Bayer 73, the 2-aminoethanol salt of Bayer 2353 (2',5-dichloro-4'-nitrosalicylanilide) in solution is not well understood. Therefore it is recommended that:
 - A. THE COMPOSITIONS OF TFM AND BAYER FORMULATIONS BE ELUCIDATED AND ANY IMPURITIES IDENTIFIED; AND
 - B. THE INTERRELATIONSHIP BETWEEN BAYER 73 AND BAYER 2353 BE EXAMINED AND THE RELATIVE CONCENTRATIONS AT AQUEOUS EQUILIBRIUM BE ESTABLISHED IN ORDER TO DETERMINE THE ACTIVE SPECIES IN SOLUTION.
2. The fate of TFM in the water column is now fairly well documented. The predominant mechanism for removal of TFM from the water column is photolytic degradation, yielding several photoproducts. Laboratory studies have demonstrated that Bayer 73 photodegrades to several products. Both TFM and Bayer 73 undergo varying degrees of sorption to sediments, the principle controlling factor being the sediment organic content. In addition, Bayer 73 precipitates from solution at pH 6.5.

Under anaerobic conditions, TFM is reduced to RTFM and there is evidence to suggest that Bayer 73 is microbially transformed. Biotransformation of TFM and Bayer 73 has been demonstrated in teleosts but has been investigated in only one species of invertebrate. TFM and Bayer 73 are not bioaccumulated in green algae, aquatic macrophytes, invertebrates (with the exception of annelids) or teleosts. Little information on biotransformation is available for amphibians and reptiles.

Although the information highlighted above is useful in attempting to assess the persistence of TFM and Bayer 73 in the aquatic environment, the Scientific Panel considers that to predict the potential exposure of aquatic organisms to TFM and Bayer 73 accurately, several pieces of critical information on the fate of lampricides in the natural aquatic environment are required. It is therefore recommended that:

THE LEVELS OF TFM PHOTOPRODUCTS BE DETERMINED UNDER FIELD CONDITIONS; THE POSSIBILITY THAT BAYER 73 PHOTODEGRADES IN THE AQUATIC ENVIRONMENT BE CONFIRMED; FOR BOTH TFM AND BAYER 73, THESE PRODUCTS SHOULD BE EVALUATED WITH RESPECT TO THEIR PERSISTENCE AND TOXICITY; FACTORS AFFECTING SEDIMENT BINDING AND INCORPORATION INTO SEDIMENTS BE EXAMINED UNDER ACTUAL FIELD CONDITIONS FOR BOTH TFM AND BAYER 73; AND THE EXTENT AND POSSIBLE ENVIRONMENTAL SIGNIFICANCE OF BIOTRANSFORMATION OF TFM AND BAYER 73 IN THE AQUATIC ENVIRONMENT BE INVESTIGATED USING REPRESENTATIVE SPECIES AND APPROPRIATE METHODS.

3. Knowledge of the biological effects of lampricides on aquatic biota is based primarily on acute lethality studies. Field observations have, in general, confirmed results obtained from acute toxicity tests conducted in the laboratory. Although they have provided much useful information on specific issues, current studies have not adequately focused on the potential long-term effects on nontarget organisms that might be caused by the repetitive use of lampricides. Much of the information collected thus far on the use of lampricides could be used to detect acute changes in the aquatic environment. However, comprehensive studies have not been undertaken that could provide critical information on the ecological importance of changes in community structure and populations of nontarget organisms because of the use of lampricides. It is therefore recommended that:

FIELD STUDIES BE DESIGNED TO INVESTIGATE THE QUALITATIVE AND QUANTITATIVE NATURE OF CHANGES CONSIDERED TO BE OF ECOLOGICAL SIGNIFICANCE IN THE NATURAL AQUATIC ENVIRONMENT AND WHICH COULD RESULT FROM THE USE OF LAMPRICIDES.

Regarding the potential effects on nontarget organisms of repeated lampricide use, the Scientific Panel feels that it is important to consider items identified in both Recommendations 2 and

3 for the design of future field studies, surveys and monitoring programs. Such investigations should be designed to consider those abiotic and biotic processes that determine potential exposures of aquatic organisms to lampricides as well as those organisms most likely to be affected by such exposures. Since natural variability may mask response to treatment, both temporal and spatial controls must be included. Pretreatment information is necessary to ensure temporal control. Spatial control may be provided by a comparison of treated and nontreated areas with similar characteristics.

CATEGORY B

1. There are inadequate quantitative data concerning the toxicity of Bayer 73 or the TFM:Bayer 73 mixture to algae, aquatic macrophytes or native species of lampreys. This information is needed to assess what effects, if any, these lampricides would have during a field treatment. Therefore it is recommended that:

THE TOXICITY OF BAYER 73 AND THE TFM:BAYER 73 MIXTURE TO REPRESENTATIVE SPECIES OF ALGAE AND AQUATIC PLANTS AS WELL AS TO INDIGENOUS SPECIES OF LAMPREYS BE ESTABLISHED IN THE LABORATORY UNDER CONDITIONS SIMULATING AN ACTUAL TREATMENT.

2. In several studies on the effects of lampricides on invertebrates, a single genus or species has been used to characterize the sensitivity of a taxonomic group. Different species of insect larvae have shown marked differences in susceptibility. In addition, aside from some observations and preliminary data concerning frog larvae, mudpuppies (Necturus sp.) and turtles, there is no quantitative field information on the toxicity of lampricides to amphibians and reptiles inhabiting the Great Lakes tributaries. Therefore it is recommended that:

A. WHERE SPECIES DIVERSITY/BIOMASS DATA FROM PRE- AND POSTTREATMENT STUDIES SUGGEST A LACK OF ADEQUATE INFORMATION, FURTHER TESTING AT THE SPECIES LEVEL BE CONDUCTED; AND

B. THE TOXICITY OF TFM, BAYER 73 AND THE TFM:BAYER 73 MIXTURE BE DETERMINED FOR REPRESENTATIVE SPECIES OF AMPHIBIANS AND REPTILES UNDER CONDITIONS SIMULATING STREAM TREATMENTS.

3. Despite the success of the lampricide treatment program in reducing the numbers of sea lampreys, there exists a possibility that sea lampreys could acquire resistance to TFM and Bayer 73 because several generations have been spawned since the treatment program began in 1958. Therefore it is recommended that:

THE POSSIBILITY OF A CHANGE IN SUSCEPTIBILITY OF SEA LAMPREYS TO LAMPRICIDES BE INVESTIGATED.

4. Although early life stages of walleyes exposed to TFM did not exhibit any adverse delayed effects, there is some question regarding possible toxic effects during early embryonic development in other species. Many species spawn during conditions unfavorable for stream treatment (e.g. high water flow and low temperatures) and therefore would unlikely be affected. However, other fish such as minnows, spawn in late spring and early summer during the treatment season and hence could be affected. Therefore, it is recommended that:

A FURTHER STUDY BE CARRIED OUT ON THE POSSIBLE EFFECTS OF TFM ON THE EARLY LIFE STAGES OF REPRESENTATIVE SPECIES OF FISH.

CATEGORY C

1. Although the specific mode of action of TFM and Bayer 73 remains undefined, the biotransformation of TFM and Bayer 73 into less toxic products has been demonstrated in teleosts. Little or no information is available concerning the biotransforming ability in invertebrates, amphibians or reptiles. Therefore it would be useful if:

FURTHER STUDIES WERE CONDUCTED TO ELUCIDATE THE MODE OF ACTION OF TFM AND BAYER 73 AS WELL AS THE BIOTRANSFORMATION ABILITY OF REPRESENTATIVE SPECIES OF INVERTEBRATES, AMPHIBIANS AND REPTILES, FOR A BETTER UNDERSTANDING OF THE SENSITIVITY OF EXPOSED ORGANISMS.

2. The bioaccumulation potential of TFM and Bayer 73 does not appear to be significant in representative species of green algae, aquatic macrophytes, invertebrates or teleosts, despite some questions regarding the experimental design of some relevant studies. The concentrations of TFM and Bayer 73 in moribund sea lamprey larvae are not known, and hence the threat to potential consumers could not be fully evaluated. Based on the oral toxicity data on birds and mammals, it is unlikely that potential consumers would be adversely affected. Nevertheless, to confirm the bioaccumulation potential in these organisms, it would be useful if:
 - A. THE BIOACCUMULATION POTENTIAL OF TFM AND BAYER 73 IN REPRESENTATIVE SPECIES OF ALGAE, AQUATIC MACROPHYTES AND INVERTEBRATES WERE EXAMINED FURTHER; AND
 - B. THE CONCENTRATIONS OF TFM AND BAYER 73 IN FRESHLY-KILLED SEA LAMPREY LARVAE WERE DETERMINED TO ASSESS THE SIGNIFICANCE OF LAMPRICIDE-CONTAINING LARVAE IN THE DIET OF POTENTIAL CONSUMERS.
3. Water temperature has little or no effect on the toxicity of either Bayer 73 or the TFM:Bayer 73 mixture to sea lampreys and teleosts.

Because no data are available for invertebrates, it would be useful if:

THE EFFECTS OF TEMPERATURE ON THE TOXICITY OF BAYER 73 AND THE TFM: BAYER 73 MIXTURE TO REPRESENTATIVE SPECIES OF INVERTEBRATES WERE EXAMINED.

4. Macroinvertebrates that appear to be very susceptible to lampricides are those that feed primarily upon small particles. This suggests that TFM bound to particulate matter may be ingested and released in the gut. Were this true, TFM could act as a gut poison. Therefore it would be useful if:
 - A. STUDIES WERE CARRIED OUT TO DETERMINE THE EXTENT TO WHICH TFM IS BOUND TO SMALL PARTICLES; AND
 - B. THE FEEDING HABITS OF INDIVIDUAL SPECIES AND THE EFFECT OF GUT pH ON ABSORPTION-DESORPTION PROCESSES OF TFM WERE INVESTIGATED.

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1.0

INTRODUCTION

1.1 SEA LAMPREY MANAGEMENT PROGRAM

TFM (3-trifluoromethyl-4-nitrophenol) is the principal lampricide currently applied to tributaries of the Great Lakes of North America under the auspices of the Great Lakes Fishery Commission. Bayer 73, also known as clonitralid, (the 2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide) is sometimes added at a rate up to 2% by weight to TFM, permitting the amount of TFM to be significantly reduced, while still maintaining selective toxicity. However, this mixture is used only as a cost-saving measure where very large quantities of TFM would otherwise be required. Bayer 73 is also used in a granular form as a bottom toxicant to survey streams and lake areas for the presence of sea lamprey larvae (*Petromyzon marinus*) and to treat lentic water bodies in Canada and New York State. TFM and Bayer 73 are licensed in Canada and the United States for restricted use by Fisheries and Oceans Canada and the U.S. Fish and Wildlife Service as well as by authorized Provincial and State fish and game employees.

Of the 5743 streams flowing into the Great Lakes, only 433 are known to have been inhabited by sea lampreys (Morman *et al.* 1980). The limited number of streams utilized by sea lampreys is thought to be related to their requirements for spawning (see Section 1.2). Physical barriers limiting the upstream movement of spawning adults make other streams unavailable. In addition, polluted streams, although sometimes used by spawning adults, are unsuitable for larval survival (Morman *et al.* 1980). Because of the long larval life span of sea lampreys, only 20-30% of infested streams are treated in any one year.

The continued applications of TFM and Bayer 73 since 1958 have significantly reduced the population of sea lampreys in the Great Lakes. This was achieved by destroying the larvae before they were able to metamorphose into the parasitic stage, which migrates from streams to the Great Lakes and feeds on fish, causing either death or a severe reduction in growth. As a result of the sea lamprey management program, sport and commercial fisheries that exceeded \$1 billion in value in 1979, have been restored (Fetterolf 1980).

1.2 LIFE CYCLE OF THE SEA LAMPREY

The life cycle of the sea lamprey was detailed by Applegate (1950) and reviewed by Howell (1966) and hence will be only briefly described here. Within the Great Lakes, mature sea lampreys (ranging in length from 30.5 to 61.0 cm) ascend streams from April until the end of July; the peak spawning period occurs in June and July. There are three basic requirements for successful spawning. Gravel in the stream bed must be from 0.5 to 5 cm in diameter, and small amounts of sand should be present. The current must be unidirectional over the nest at a

velocity of 0.3 to 1.7 m.sec⁻¹. The water temperature should be between 15.6 and 21°C. Piavis (1961) determined the optimum temperature for egg development to be 18.3°C. More recently, it has been shown that eggs spawned at 10.0 to 11.7°C will develop, although more slowly, with survival of the embryos ranging from 85-97% (Hanson and Manion 1978).

Generally, the male initiates nesting and the female joins soon after. The nest is built in a horseshoe-shaped ridge with the open end facing upstream. The female anchors herself to a large stone in the upstream portion of the nest with the aid of the oral disc. The male then attaches himself to the female in the branchial region with his mouth and wraps his body around the female so that their genital pores are close together. Eggs and milt are released simultaneously by vibrating and contracting their bodies. Spawning occurs at 5-10 minute intervals until the fish are spent, usually in less than 24 hours. After each spawning act of roughly 2-5 seconds, the adults resume nest building. The current carries the nonbuoyant, adhesive eggs into the crescent-shaped ridge, where they are subsequently covered with sand dislodged by the nest-building activity. The adults die within a few days after spawning. Those adults that do not spawn also die. In both cases, death results from the complex interaction of several factors including the degeneration of the internal organs, a breakdown of regulatory mechanisms, lack of essential substances and the accumulation of poisonous metabolites (Larsen 1980).

Manion and Hanson (1980) estimated that on the average, each spawning female produced about 60 000 eggs, of which only 14% were likely to be deposited within the nest. The rest were apparently carried downstream by the water current or swept out by the adults as they continued nest building. The eggs that remain in the nest have a survival rate approaching 90%.

About 13 days after spawning the eggs hatch. Larvae remain in the nest an additional 7 days and undergo further development. The larvae, which average 6.4 mm in length, then leave the nest and drift downstream until they reach an area of slow-moving water with a soft bottom, into which they burrow. Their sedentary larval life usually lasts 4 to 7 years (but has been observed to reach up to 18 years (Manion and Smith 1978)) and during that time, they feed on microorganisms, especially diatoms, by filtering water taken in during respiration.

Upon attaining a critical size (length), the larvae undergo a period of metamorphosis during which a number of morphological and physiological changes occur (Youson 1980). This transformation requires several weeks and generally occurs between mid-July and the end of September. Obvious external changes include the development of functional eyes and a tooth-lined, circular sucking disc with a rasping tongue. In addition, the dull brown color changes to blue-gray dorsally and silvery-white ventrally.

After metamorphosis, the lampreys leave their natal streams and enter the Great lakes. This takes place from late October to early May. Peak movements occur during periods of high water flow, e.g. during fall rains and spring snow melt. In the lakes the lampreys attach themselves to host fish by means of the oral sucker disc and feed on blood and body fluids. The parasitic feeding interval lasts from 12 to 20 months, during which time the adults increase in length from 15 to 45 cm on the average. They then return to the streams and spawn.

1.3 HISTORICAL USE OF LAMPRICIDES

Sea lampreys were first reported in Lake Ontario in 1835 (Lark 1973). It is a matter of conjecture whether sea lampreys were native to Lake Ontario or whether they reached Lake Ontario from the sea through canal systems constructed in the early 1800's (Lamsa *et al.* 1980). Niagara Falls was a natural barrier that blocked fish movement to the other Great Lakes until the Welland Canal was built in 1829. It provided a bypass that linked Lake Ontario to Lake Erie (Pearce *et al.* 1980), and the sea lampreys then spread throughout the Great Lakes. They were first noted in Lake Erie in 1921 (Dymond 1922), Lake Huron in 1937 (Applegate 1950), Lake Michigan in 1936 (Hubbs and Pope 1937) and Lake Superior in 1938 (Smith and Tibbles 1980). They may have been present in some of the lakes before these observations were made (Smith and Tibbles 1980).

Although sea lampreys were reported to have attacked fish in Lake Ontario about 1890, they were not considered a threat to lake trout (*Salvelinus namaycush*) production (Dymond *et al.* 1929). Only after extremely heavy fishing pressure had depressed both the abundance and size of fish in the population, were sea lampreys thought to have become a factor in the decline and eventual extinction of the lake trout from Lake Ontario (Christie 1973).

Predation on fish populations by sea lampreys has not been considered a problem in Lake Erie in the past, perhaps because this lake has only a few suitable streams for spawning. Up to the early 1970s, sea lamprey populations in Lake Erie had not become abundant (Hartman 1973).

In the upper Great Lakes (Lakes Huron, Michigan and Superior), there have been drastic effects on fish populations. Whether these effects were due solely to sea lampreys or to sea lampreys acting in concert with intensive fishing pressure and habitat degradation remains a matter of controversy. Some authors have ascribed the loss of the lake trout solely to the actions of sea lampreys (Hile 1949; Hile *et al.* 1951; Eschmeyer 1957; Wells and McLain 1973). Further evidence for this contention was provided by data on a lake trout population in South Bay, Lake Huron. Fry (1953) found that the native stock disappeared after sea lampreys arrived, even though the population was not fished

commercially. Furthermore, none of the fish planted in subsequent years survived to age VII (Fry and Budd 1958; Budd and Fry 1960). The incidence of sea lamprey wounds on lake trout greater than age IV increased rapidly, along with an increase in the rate of mortality.

Other authors have concluded that overfishing, in combination with sea lamprey predation, was responsible for the decline of fish populations (Smith 1968; Lawrie and Rahrer 1972, 1973; Berst and Spangler 1973; Pycha and King 1975). For example, according to Pycha and King (1975) the lake trout abundance in Lake Superior gradually decreased between 1929 and 1949. In the late 1940s and early 1950s, intensive fishing, aided by the introduction of the nylon gill net, was thought to have been largely responsible for the decline of the lake trout population. In the early to mid-1950s, sea lampreys apparently became a serious predator, and, together with overfishing and habitat degradation, contributed to the demise of the lake trout population.

Despite these opposing viewpoints, it is important to note that in both cases, sea lampreys were considered to have played a significant role in the severe reduction of fish populations. The effects have been detailed by Pycha and King (1975) and Smith (1968, 1971) in the upper Great Lakes. In general, sea lampreys first attacked lake trout and the only other deepwater predatory species, the burbot (Lota lota). At the outset, the loss of these predators favored an increase of their primary prey, seven species of ciscoes (Coregonus spp.). Sea lampreys then turned to the ciscoes, eliminated the two largest species, Coregonus johannae and Coregonus nigripinnis, and severely reduced the abundance of four other species, thereby enabling the bloater, Coregonus hoyi to become abundant. The lampreys also attacked whitefish (Coregonus clupeaformis), catostomids (Catostomus spp. and Moxostoma spp.) and rainbow trout (Salmo gairdneri). During the late 1940s, the alewife (Alosa pseudoharengus) entered Lakes Huron and Michigan and thrived, evidently because of the absence of predators. The alewife adversely affected populations of several native species including the bloater, lake herring (Coregonus artedii), yellow perch (Perca flavescens) and emerald shiner (Notropis atherinoides). However, the alewife never became abundant in Lake Superior, apparently because the water was too cold.

In response to the declining lake trout fishery, the Great Lakes Sea Lamprey Committee was established in 1946 to coordinate early research and control activities (Smith 1971). In the interval from 1946-1949, studies were conducted concerning the effects of lampreys on commercial fisheries, and surveys were carried out to identify lamprey spawning streams. Canada and the United States ratified the Convention on Great Lakes Fisheries in 1955, thereby establishing the Great Lakes Fishery Commission, which began operations in 1956. One of its first activities was the formulation of a program to eradicate or minimize the sea lamprey population. The control program continues to be directed and coordinated by the Commission and is presently contracted to the

Department of Fisheries and Oceans Canada and the U.S. Fish and Wildlife Service.

A study by Applegate (1950) suggested that three stages in the life cycle of the sea lamprey would be amenable to control. First, the spawning migration of adults could be blocked before they could reach the spawning areas. This approach was tried initially, despite the shortcoming that a reduction in numbers would not become apparent for several years. This delay would occur because many generations of larvae would already be present in the streams and all would have to pass through the parasitic stage before returning to spawn. In 1950-1951, 31 screen-type weirs were installed together with one electrical screen on tributaries of the upper Great Lakes to block upstream migrations. Because these mechanical barriers were expensive to build, operate and maintain and because of their frequent failures during ice breakup and flooding, they were discontinued. A system consisting of electrical barriers and mechanical traps was then developed. This proved to be cheaper and operated at a greater efficiency. However, it too suffered from numerous drawbacks. The barrier network was incomplete, i.e. barriers were placed only on a limited number of streams used by sea lamprey spawners, and many barriers were operated only 1 to 2 years before being removed. Consequently, it was questioned whether this system had much effect on the total population of sea lampreys. Nevertheless, some barriers were operated over several years providing useful information concerning the length, weight, sex composition and time of migration of sea lampreys. These data were later used to assess the effect of the lampricide program.

The second stage in the life history considered to be susceptible to control was that of recently metamorphosed individuals during their downstream migration. However, stopping them from leaving the streams was not considered feasible because the extended migration period can occur throughout the winter when stream operations are difficult.

The third possible alternative was to destroy the ammocoetes (larvae) while they were in the stream. This approach was particularly attractive since all generations present would be eliminated at the same time, resulting in immediate control. While the electrical barrier system was being developed, a search for a chemical lampricide was initiated. At the Hammond Bay Biological Station on Lake Huron, approximately 6000 chemicals were screened by the U.S. Fish and Wildlife Service. Of these, 10 halogenated nitrophenols were eventually identified that were more toxic to sea lampreys than to teleost fish. Because of its selective toxicity, physical and chemical properties that facilitated application in the field, high effectiveness at low concentrations and reasonable cost, 3-trifluoromethyl-4-nitrophenol (TFM) was selected for use.

TFM was originally tested in 1958 in static bioassays using larval sea lampreys and juvenile rainbow trout to determine the effects of temperature, pH, alkalinity and hardness on its biological activity. Further testing using flow-through raceways to approximate actual stream conditions was then carried out. A number of different species of fish as well as crayfish, turtles and caddisfly larvae were exposed (Applegate *et al.* 1961). Since TFM was found to be selectively toxic, it was decided to treat three tributaries of Lake Superior, which were known to be infested with sea lamprey larvae and which differed greatly in physical characteristics. The Great Lakes Fishery Commission chose to emphasize Lake Superior for controlling the sea lamprey population since lake trout had virtually disappeared from Lakes Michigan and Huron, whereas some native stocks were still present in Lake Superior (Smith 1971). In all three field trials, sea lamprey larvae were killed in large numbers. Eight other Lake Superior streams were also treated in 1958. As lampricide use increased, electrical barriers were phased out as control devices, except for a small network retained for assessment purposes. These barriers indicated a sharp decrease in the population of sea lampreys (see Section 8.3.1) following the onset of chemical control. The use of TFM was then expanded to Lakes Michigan and Huron in 1960. Because of financial constraints and the desire to expand treatments on Lake Michigan tributaries, the control program on Lake Huron was abandoned between 1963 and 1965, before being resumed in 1966. In 1971, treatments began in Lake Ontario, after the apparent failure of a salmonid stocking program between 1968 and 1971 because of sea lamprey predation (Pearce *et al.* 1980). By 1973, both the number of fish bearing sea lamprey attack marks and the wounding rate per fish had declined substantially, while the average size of fish had increased. However, the incidence of scarring remained relatively high, suggesting that recruitment from other areas was occurring. Parts of the Oswego river drainage, which connects to Lake Ontario, were suggested as the possible source of recruitment, since the system was known to harbor populations of sea lampreys (Pearce *et al.* 1980).

Because the few sea lampreys in Lake Erie have not posed a threat to fish populations in the past, TFM treatments have not been initiated (Hartman 1973). However, continued improvements in the water quality of tributary streams, as a result of pollution control measures, are making them suitable for spawning. Numbers of lamprey spawners are increasing, as are sea lamprey marks on planted lake trout and other salmonids. Consequently, the use of lampricides in streams tributary to Lake Erie is currently being considered (Lamsa 1984).

Despite the success of TFM treatments in reducing sea lamprey abundance (Smith 1971; Smith *et al.* 1974), the screening of selectively toxic chemicals continued and in 1963 the molluscicide Bayer 73 was found to be highly toxic to sea lampreys in mixtures with TFM, as well as alone. When Bayer 73 was added to TFM at a rate of 0.5 to 2.0% by weight, the amount of TFM required to effect the same degree of kill was reduced by up to 50%; yet the mixture was still selectively toxic to sea

lampreys (Howell et al. 1964). Further study led to the development of Bayer 73 in a heavy granular formulation as a bottom toxicant for use in surveying and treating lentic bodies of water. Presently, TFM or a mixture of TFM and Bayer 73 are used to treat entire reaches of Great Lakes tributaries whereas 5% granular Bayer 73 is used only to survey and treat slow-moving waters (see Section 3.2).



2.0

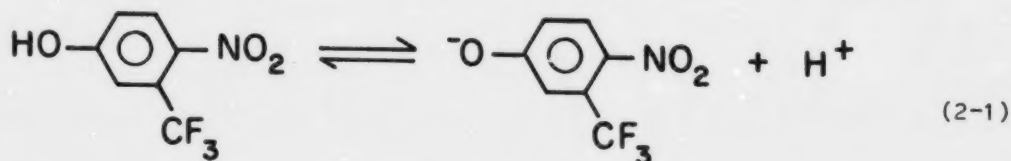
CHEMISTRY OF TFM AND BAYER 73

2.1 TFM

2.1.1 PHYSICAL-CHEMICAL PROPERTIES

TFM, a halogenated mononitrophenol having the molecular formula $C_7H_4F_3NO_2$, and a molecular weight of 207.11, is a pale yellow crystalline solid that melts at 75-76°C (Smith *et al.* 1960).

The sodium salt of TFM has a solubility in water of 5 g.L⁻¹ at 25°C (Daniels *et al.* 1965). The free phenol is very soluble in a variety of organic solvents including N,N-dimethylformamide (DMF), benzene, toluene, acetone, methanol, ethanol and ethyl ether. Aqueous solutions of TFM are acidic (pK_a 6.07±0.03) (Smith *et al.* 1960) because of the equilibrium between the free phenol and phenolate anion:



The free phenol is colorless (acidic solution) whereas the anion is deep yellow (alkaline solution); this color change forms the basis of the colorimetric analysis of TFM in water (see Section 4.1.1). The pK_a of TFM in water has been recently estimated, using the Hammett relation, to be 6.38 (Chantooni and Kolthoff 1976, 1979). The pK_a s of TFM in a variety of nonaqueous solvents are detailed in Table I-1 of Appendix I.

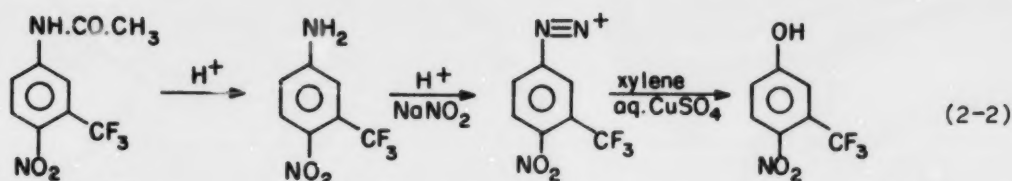
2.1.2 SPECTRAL PROPERTIES

The maximum absorption of light by TFM occurs at 290 nm in acidic solutions and at 395 nm in basic solutions. The change in ultraviolet-visible light absorption with respect to pH is illustrated in Figure I-1 of Appendix I. Infrared, electron spin resonance and mass spectra of TFM have been recorded (see Appendix I).

2.1.3 SYNTHESIS AND FORMULATION

TFM is formed as a side product in the preparation of nitro-trifluoroacetanilide (DeBrouwer 1930) and 2,5-dihydroxybenzotrifluoride (Whalley 1949); however, the only commercially important route to its synthesis appears to be via diazotization of 3-trifluoromethyl-4-nitroaniline (U.S. Rubber Co. 1963). This amine is formed in the acid hydrolysis of the corresponding acetanilide and the resulting acidic solution can be used directly in the conversion of the aniline to the

phenol. High yields in this latter reaction are achieved through the use of a boiling mixture of xylene and aqueous copper sulfate. The overall route is:



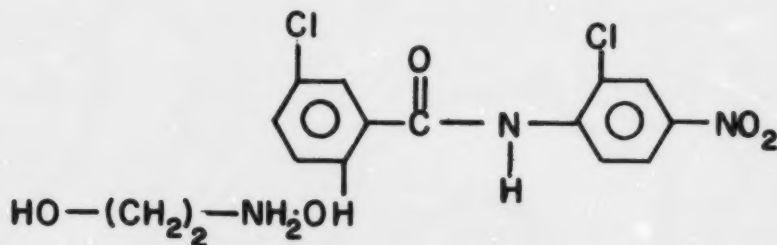
The products of this reaction are said to contain 90% TFM (Schnick 1972). However, Daniels *et al.* (1965) refer to an analysis of technical TFM which revealed that it was composed of 70% 3-trifluoromethyl-4-nitrophenol and 20% of other isomers, primarily 3-trifluoromethyl-6-nitrophenol.

The preparation of 3-trifluoromethyl-4-aminophenol (RTFM) has been reported by Lech (1973a) and Carey and Fox (1981). A discussion of physical-chemical properties, such as infrared spectra, pK_a , ultraviolet-visible spectra, absorption maxima, molar extinction coefficients and chromatographic isolation may be found in Lech (1973a). Tritium-labelled TFM has also been prepared (Lech and Moyer 1972).

2.2 BAYER 73

The correct common name for Bayer 73 is clonitralid and for Bayer 2353 niclosamide. Bayer 73 is the product code of the manufacturer. However, since it has always been referred to as Bayer 73 by agencies involved in the sea lamprey management program, it will be termed Bayer 73 in this document.

The structure of Bayer 73, the 2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide (Bayer 2353), is given below:



It is a yellow powder with a molecular weight of 388.21 and a melting point of 216°C. Bayer 73 is only slightly soluble in water, having a solubility of $230 \pm 50 \text{ mg.L}^{-1}$ at 25°C (Luhning *et al.* 1979). As the pH drops below 7.0, it becomes insoluble (Meredith 1971a; NFRL 1983a). The UV absorption maximum occurs at 330 nm in a methanol:0.01 mol.L⁻¹ acetate buffer (87:13, v:v) (Dawson 1982). The mass spectrum of Bayer 2353 is illustrated in Figure I-4 of Appendix I.

2.3 ANALYTICAL VS FORMULATED GRADE LAMPRICIDES

In field treatments, formulated grade TFM (~35% active ingredient) is used. The remaining 65% of the formulation is made up of inert materials, principally the carrier substance, dimethylformamide and water. In laboratory studies, analytical grade TFM is often employed ($\geq 95\%$ active ingredient). All concentrations reported in this document are based on the active ingredient.

Similarly for Bayer 73, which is used either as a 70% active wettable powder in mixtures of TFM or in a 5% active granular formulation, all concentrations are based on the active ingredients involved.



3.0

USE PATTERNS

3.1 REGISTRATION STATUS

The use of TFM and Bayer 73 as lampricides has been approved by the U.S. Environmental Protection Agency (EPA) and by the Federal Interdepartmental Committee on Pesticides chaired by Agriculture Canada. In the United States, this approval carries EPA registration numbers 6704-45 for TFM, 3125-136 for powdered Bayer 73 and 3125-219 for granular Bayer 73. Recently, a Special Need Registration No. NY-820011 (EPA No. 3125-219) was issued authorizing use of 5% granular Bayer 73 as a lampricide in New York State. In Canada, approval is provided under Agriculture Canada registration numbers PCP 11763 for TFM, PCP 10842 for powdered Bayer 73 and PCP 10841 for granular Bayer 73. The EPA has ruled that dimethylformamide (DMF), the primary inert ingredient in the liquid formulation of TFM, is exempt from a requirement for the establishment of a tolerance (Federal Register 1982). It has been shown that DMF is devoid of mutagenic or carcinogenic properties (Antoine *et al.* 1983). All of the current methods of application and uses of TFM and Bayer 73 involved in sea lamprey control by the U.S. and Canadian agents are in compliance with the approved labels given in Appendix II.

3.2 TREATMENT TECHNIQUE

3.2.1 THE USE OF TFM, BAYER 73 AND THE MIXTURE

TFM (field grade ~ 35% active ingredient) may be used either in combination with Bayer 73 (70% wettable powder (W.P.)) or alone to treat lamprey-infested streams. The addition of Bayer 73 at the rate of 0.5-2% of the TFM can limit the treatment cost by reducing the total lampricide requirement by up to 50%. Although this mixture is still selectively toxic, it is more toxic to both lampreys and nontarget organisms than TFM alone. Bayer 73 (70% W.P.) is almost as toxic to rainbow trout as it is to the lamprey (Howell *et al.* 1964) and therefore is not used alone for treatment purposes. In deciding whether or not to use the mixture, one must weigh the potential savings in the amount of lampricide required against the increased risk to nontarget organisms and the added complexity of treatment. In complex stream systems with low to medium discharges or where local conditions and the presence of highly sensitive fish species (see Section 11.1.1) would create an unacceptable risk, the choice is usually made to treat with TFM alone. Generally, the mixture is used only in the treatment of large rivers or where high concentrations of TFM are necessary (i.e. where large quantities of TFM are required).

Granular Bayer 73 (5% active ingredient) is employed most frequently by control units in Canada and the United States as a survey tool to detect and collect ammocoetes. It settles to the bottom,

releases the toxicant and produces an initial irritating effect, which causes the animals to leave their burrows within 1 to 2 hours and swim to the surface where they may be readily observed or captured with hand nets. In addition, when lamprey-infested tributaries contain areas of slow-moving water (e.g. bays, estuaries and backwaters), or where thermal stratification prevents the warmer treated surface water from mixing with the hypolimnetic water, making TFM largely ineffective, granular Bayer 73 is also used. This control practice is carried out only in Canada and New York State. Granular Bayer 73 is not sanctioned for general use as a lampricide in the rest of the United States. Nevertheless, when Bayer 73 is used as a survey tool many of the larvae will have received a lethal dose by the time they leave their burrows (Tibbles 1967). Despite the fact that treatment with granular Bayer 73 is less effective than treatment with TFM, repeated treatments of the same areas at intervals of a year or more, have generally resulted in diminishing densities of ammocoetes.

3.2.2 PREPARATIONS AND PROCEDURES

The preparations and procedures for treating lamprey-infested streams are described in detail in Smith *et al.* (1974) and will only be outlined here. The stream treatment season normally lasts from early May to late October. Winter conditions make treatment logistically unfavorable. The high runoff associated with late fall and early spring not only increases the total quantity and cost of lampricides unnecessarily, but also makes it difficult to maintain the desired concentrations during the treatments because of fluctuations in water levels. Normally, the annual treatment program begins in the southern part of the Great Lakes Basin, since stable flow conditions occur there first.

Before treating any stream, a survey is carried out to assess the need for treatment. Using either an electroshocker (in shallow water over a small area) or granular Bayer 73 (in deep water, low conductivity water and over large areas) the location, size and density of the ammocoetes are determined. Lamprey larvae have been shown to metamorphose once they attain a certain size. For example, in Lake Superior, larvae begin transforming at a length of 120 mm. Therefore, if surveys reveal the presence of ammocoetes over 100 mm in length, a lampricide treatment is usually scheduled for the subsequent year.

Immediately prior to treatment, 24-hour bioassays are performed to determine the range of concentrations of lampricide that will kill free-swimming lamprey larvae without causing significant fish mortality. These assays use stream water since water chemistry can affect the toxicity of TFM (see Section 7.0). Initially, larvae of both sea lampreys and native species were used (Smith *et al.* 1974). With the success of the lampricide program however, sea lamprey ammocoetes became increasingly difficult to obtain and hence, larvae of native species

were used (HBBS 1983). Today, only sea lamprey larvae are used as they are more susceptible to TFM than native species. The concentration range is bounded by the minimum effective concentration for lampreys (lamprey LC_{50}) and the maximum allowable concentration for fish (rainbow trout LC_{50} in the United States; brook trout LC_{50} in Canada). In some cases, where only warmwater species appear to be present, small white suckers (Catostomus commersoni) are used. In practice, a value midway between the two is usually selected as the treatment concentration. Recently, regression curves have been developed based on data accumulated over the years relating lethal concentrations to the water chemistry. These are now being used increasingly instead of the time-consuming bioassay procedure.

The duration of each treatment is usually chosen with reference to the length and complexity of the system to be treated. A stream discharge measurement is made in each tributary to be treated, and from this value and the concentration range selected, the application rate can be determined.

Since the effective application of lampricides (i.e. TFM with or without Bayer 73 W.P.) to flowing waters requires that the concentrations be maintained within narrow limits for periods typically from 8 to 20 hours, one or more controllable, electrically-driven pumps are normally employed to add the material(s) to stream water. Among the pumping systems used by the control units are variable-stroke piston and diaphragm pumps, and controllable-speed peristaltic pumps. When Bayer 73 W.P. is applied as an additive in stream treatments with TFM, the two materials may be mixed together prior to being pumped into the stream (the Canadian control unit's method), or pumped separately (the United States control unit's method). When Bayer 73 W.P. is added separately, it must, of course, be first prepared as an aqueous suspension of known concentration. When Bayer 73 W.P. is mixed directly with the TFM formulation, it loses its dispersant properties and tends to settle, unless it is kept continually agitated. Therefore, to maintain a uniform mixture of the two materials, one must stir the mixture continually during an application. The lampricide(s) is (are) normally mixed initially with stream water in a discharge manifold before being distributed through a perforated pipe stretched across the stream.

For treating very small streams and tributaries of larger streams, drip feeders operating on a constant head-syphon principle are often used. Typically, these units consist of plastic containers fitted with commercially manufactured regulating valves. Lampricide feeders are operated continuously during the treatment periods (8 to 20 h), resulting in a block of lampricide-treated water which moves downstream as it progresses. On long rivers the block may take several days to reach the mouth. In stream systems requiring application at several sites, the starting times are adjusted to achieve the coincident arrival of treated waters at any confluences. In lentic waters containing sea lamprey larvae and more or less isolated from the main stream, granular

Bayer 73 is applied by hand spreader or gasoline-powered "Solo Blowers". When ammocoetes inhabit large delta areas of lakes near stream mouths, granular Bayer 73 can be applied using crop dusting planes. In the United States, Bayer 73 is applied at a rate of 5.6 to 8.4 kg.ha⁻¹, while in Canada the application rate is 11.2 kg.ha⁻¹ (active ingredient). Theoretically, these rates should result in concentrations in the bottom 5 cm of water of 11.5 to 17.2 mg.L⁻¹ in the United States and 23 mg.L⁻¹ in Canada. However, based on an unpublished study by the U.S. Fish and Wildlife Service in La Crosse, Wisconsin (cited in Gilderhus and Johnson (1980)), in practice less than 40% of the active ingredient is released over a 24-h period.

The amount of TFM applied at each point on a stream is that which will maintain a concentration lethal to lampreys downstream to the next application point, without exceeding the maximum allowable concentration. Hence the areas immediately downstream are often exposed to much higher concentrations of the chemical than other areas farther downstream. The minimum effective and maximum allowable concentrations employed differ considerably in the tributaries of the Great Lakes and in the lakes themselves. In general, the range of concentrations of TFM used in the Great Lakes is from 1 to 17 mg.L⁻¹ (Dykstra and Lennon 1966). For example, the minimum effective and maximum allowable concentrations in Lake Superior are typically 1 to 3, and 3 to 9 mg.L⁻¹ respectively, whereas in Lake Huron the respective levels are 6 and 16 mg.L⁻¹ (Smith and Braem 1976).

The concentrations of TFM in treated water in Canada are monitored using the colorimetric method described by Applegate *et al.* (1961). In the United States, TFM and Bayer 73 are determined by high performance liquid chromatography (HPLC) and gas liquid chromatography (GLC) respectively. This is presently changing towards a system where both lampricides are measured simultaneously by HPLC (see Section 4.1). Bayer 73 is not measured in Canada. Water samples for analysis are taken at preselected stations between the application points and the stream mouth at intervals of 1 to 2 hours. Occasionally the lampricide concentrations may fall below the minimum lethal threshold as a result of adsorption, degradation and/or dilution. To correct for this, additional lampricide may be added by means of a booster feeder located just above the area of critical concentration.

Sea lamprey larvae may not be reestablished for several years, particularly in small streams. Retreatment of a given stream depends upon if and when sea lampreys recolonize the stream. In most cases sea lamprey larvae require four years to reach the age of metamorphosis, although in favorable streams of the lower lakes basin, they may require only three years. Stream surveys are therefore vital in determining the need to retreat.

3.3 QUANTITIES OF LAMPRICIDES APPLIED TO TRIBUTARIES OF THE GREAT LAKES

The quantities of TFM and Bayer 73 (both as a wettable powder and as a granular formulation) that have been added to the tributaries of Lakes Superior, Michigan, Huron and Ontario in the United States and Canada since 1958 (when control operations began) until 1983 are listed in Tables 3-1, 3-2, 3-3 and 3-4 respectively. The amounts of granular Bayer 73 employed in the United States are those used in stream surveys in conjunction with treatments. In Canada, the amounts of Bayer 73 applied are those used in treatments with TFM. The quantities of granular Bayer 73 used in Canada during surveys are being compiled and are not available at the present time (Johnson 1984).

The magnitude of the annual treatment program varies considerably depending on the number of streams treated and their physicochemical characteristics. For instance, the amount of lampricide used in each stream is influenced by discharge rate, complexity of the stream system, water chemistry and attenuation of the toxicant.

Between 1958 and 1983, a total of 672 treatments (224 in Canada and 448 in the United States) have been carried out on 123 Lake Superior streams, in which a total of 436 695 kg of TFM has been applied. During the same period, 3011 kg of powdered Bayer 73 and 1113 kg of granular Bayer 73 have also been used.

The number of streams treated and quantities of TFM used in Lake Michigan between 1960 and 1983 are similar to those of Lake Superior. A total of 477 921 kg of TFM have been used in 442 treatments of 116 streams, in the United States, while in addition, 1324 and 391 kg of powdered and granular Bayer 73 have been applied respectively.

Fewer stream treatments have been conducted in Lake Huron than in Lakes Superior and Michigan, largely because no tributaries were treated between 1963 and 1965. A total of 376 treatments (174 in Canada and 202 in the United States) using 286 504 kg of TFM have been carried out in 99 streams up to and including 1983. The amounts of Bayer 73 used in the powdered and granular form were 1412 and 524 kg, respectively.

Treatments of 48 tributaries to Lake Ontario began in 1971 in Canada and one year later in the United States. The quantity of TFM used in 166 treatments (88 in Canada and 78 in the United States) totalled 63 519 kg while the amounts of powdered and granular Bayer 73 applied were 257 and 324 kg, respectively.

Table 3-1. Summary of the amounts of lampricides (active ingredient) used to treat streams tributary to Lake Superior from 1958 to 1983 (from Johnson 1984).

Year	United States						Canada					
	Total No. of stream treatments	Total discharge at mouth (all treatments) (m ³ s ⁻¹)	Total stream kilometers treated ^a	No. of stream treatments using Bayer 73 ^b	TPM (kg)	Bayer 73 (kg)	Total discharge at mouth (all treatments) (m ³ s ⁻¹)	Total stream kilometers treated	No. of stream treatments using Bayer 73 ^b	TPM (kg)	Bayer 73 (kg)	Powder Granules ^c
1958	10	17.5	286	-	2 842	-	1.6	39	-	105	-	-
1959	29	42.3	860	-	8 648	-	17.1	85	-	2 665	-	-
1960	15	103.4	639	-	23 371	-	162.1	336	-	28 783	-	-
1961	7	12.7	224	-	4 395	-	21.0	97	-	3 754	-	-
1962	19	48.4	589	-	10 392	-	137.1	510	-	19 174	-	-
1963	26	86.3	642	0	0	0	113.4	290	3	14 935	223	0
1964	25	86.3	817	4	36	0	212.3	818	9	26 803	354	0
1965	16	25.1	344	8	101	0	104.1	241	8	11 075	223	0
1966	5	13.1	161	1	2 907	0	90.1	283	13	11 880	295	0
1967	17	78.4	489	7	34	18	3.3	40	4	334	2	9
1968	2	9.4	156	1	3 485	0	23.3	200	7	835	52	8
1969	19	49.4	341	11	7 870	3	33.5	77	5	3 451	57	2
1970	10	31.5	399	4	4 824	8	121.1	124	10	15 916	352	1
1971	18	93.5	581	8	15 367	31	21.1	47	5	2 794	51	18
1972	24	65.1	314	9	30	58	56.9	270	3	6 320	113	2
1973	23	92.6	676	12	13 861	37	32.0	114	3	4 257	75	2
1974	18	26.2	323	11	84	11	9.9	45	3	920	12	8
1975	27	86.5	707	17	11 157	13	136.8	192	7	15 449	293	3
1976	17	19.0	169	11	3 872	2	14.8	84	5	1 995	31	1
1977	24	39.0	501	13	6 975	5	65.3	249	5	6 665	87	5
1978	19	66.0	644	8	8 193	2	40.6	69	5	4 475	64	1
1979	11	24.8	288	3	3 043	0	175	350	6	12 340	179	102
1980	19	64.5	542	6	11 198	6	20.8	40	2	1 996	17	46
1981	13	38.7	448	NA ^d	6 338	NA ^d	99.3	113	3	9 820	131	21
1982	20	29.2	323	NA ^d	4 164	0	78.3	195	3	5 577	53	5
1983	15	40.0	272	1	8 940	22	126.5	145	5	12 396	177	1
TOTAL	448	1231.1	10 895	240	886	886	1917.5	4709	224	222 674	2771	227

^a Bayer 73 was developed and first used in 1963.

^b Granules used during surveys in conjunction with treatments.

^c Granules used in treatments with TPM

^d NA - not available.

Table 3-2. Summary of the amounts of lampricides (active ingredient) used to treat streams tributary to Lake Michigan from 1960 to 1983 (from Johnson 1984).

Year	Total No. of stream treatments	Total discharge at mouth (m ³ .s ⁻¹)	Total distance treated (km)	TFM (kg)	No. of stream treatments using Bayer 73 ^a	Bayer 73 (kg)	
						Powder	Granules ^b
1960	7	4.0	113	794	-	-	-
1961	26	31.0	406	11 199	-	-	-
1962	8	11.9	550	6 882	-	-	-
1963	22	41.4	563	11 397	6	106	0
1964	27	139.2	2 449	45 625	11	96	0
1965	24	91.7	1 384	28 505	1	14	0
1966	12	23.2	814	7 629	0	0	0
1967	12	39.6	666	14 118	2	30	0
1968	10	100.9	1 011	29 350	2	54	1
1969	12	80.6	801	19 167	6	42	62
1970	24	53.0	410	10 709	6	50	23
1971	24	58.8	1 349	16 844	7	25	70
1972	18	102.3	999	29 585	7	60	22
1973	21	100.4	1 283	23 513	9	63	43
1974	22	161.3	1 405	31 184	11	196	24
1975	19	27.0	336	6 884	11	22	0
1976	15	137.5	1 135	34 859	8	119	7
1977	37	114.9	1 181	27 752	7	99	41
1978	24	114.2	708	23 511	4	88	14
1979	18	120.8	1 398	24 391	4	56	19
1980	7	35.5	682	11 008	3	23	1
1981	23	90.8	502	12 856	6	77	43
1982	13	157.6	811	31 135	2	95	21
1983	17	72.7	874	19 024	3	65	0
TOTAL	442	1910.3	21 830	477 921	116	1324	391

^a Bayer 73 was developed and first used in 1963.

^b Granules used during surveys in conjunction with treatments.

Table 3-3. Summary of the amounts of lampricides (active ingredient) used to treat streams tributary to Lake Huron from 1960 to 1983 (from Johnson 1984)^a.

Year	United States						Canada					
	Total No. of stream treatments	Total discharge at mouth (all treatments (m ³ ·s ⁻¹))	Total stream kilometres treated	TFM (kg)	No. of stream treatments using Bayer 73 ^β	Bayer 73 (kg) Powder Granules ^γ	Total discharge at mouth (all treatments) (m ³ ·s)	Total No. of stream treatments	Total stream kilometres treated	TFM (kg)	No. of stream treatments using Bayer 73 ^β	Bayer 73 (kg) Powder Granules ^δ
1960	0	-	-	-	-	-	23.3	7	103	2 342	-	-
1961	1	0.3	23	144	-	-	74.0	14	365	16 928	-	-
1962	2	2.3	61	1 412	-	-	-	0	-	-	-	-
1966	18	24.3	703	11 550	1	9	10.6	10	204	2 495	6	41
1967	14	12.9	304	5 187	4	20	133.3	13	293	13 002	9	206
1968	4	11.3	299	5 497	1	15	36.6	11	299	8 378	8	130
1969	3	19.7	744	7 986	3	68	20.1	5	58	2 769	5	26
1970	27	58.3	499	17 921	10	123	21.4	11	195	4 350	5	55
1971	6	22.6	340	9 632	0	0	93.7	9	291	19 177	7	103
1972	12	26.5	1 399	12 273	7	44	96.8	14	262	10 862	13	136
1973	10	53.1	190	9 500	7	28	22.7	7	87	4 181	6	12
1974	13	8.2	130	2 899	6	8	179.3	9	140	2 872	5	13
1975	10	36.4	824	11 670	5	29	67.7	8	119	4 166	4	53
1976	5	43.7	200	8 147	2	27	27.6	9	127	2 636	5	31
1977	14	12.0	377	4 780	0	0	12.8	6	153	1 770	5	11
1978	15	47.9	360	16 725	1	34	26.8	11	56	2 206	3	4
1979	10	30.1	348	11 106	3	15	84.0	8	183	6 919	6	88
1980	9	54.0	171	10 525	2	50	26.5	6	89	2 283	0	0
1981	8	15.8	531	3 814	1	4	24.0	4	82	1 180	1	0
1982	10	18.6	229	5 539	1	8	7.5	8	37	956	1	0
1983	11	87.6	235	16 555	2	20	67.0	4	63	4 170	1	57
TOTAL	202	585.6	7 907	172 862	55	445	1055.7	174	3206	113 642	90	967
												306

^a No stream treatments from 1963 to 1965 (inclusive).

^β Bayer 73 was developed and first used in 1963.

^γ Granules used during surveys in conjunction with treatments.

^δ Granules used in treatments with TFM

Table 3-4. Summary of the amounts of lampricides (active ingredient) used to treat streams tributary to Lake Ontario from 1971 to 1983 (from Johnson 1984)^a.

Year	United States						Canada					
	Total discharge at mouth (all treatments) (m ³ ·s ⁻¹)	Total Stream kilometres treated	TFM (kg)	No. of streams treated using Bayer 73	Bayer 73 (kg)							
					Powder Granules ^a							
1971	-	-	-	-	-	-	23	377	8 309	12	58	69
1972	59.7	352	6 337	0	0	0	-	-	-	-	-	-
1973	7.6	107	1 405	1	0	1	3	31	726	3	7	70
1974	-	-	-	-	-	-	5	9.6	3 524	5	23	52
1975	48.1	217	4 114	4	22	1	5	8.5	2 776	4	4	25
1976	4.3	75	836	1	0	6	8	5.3	1 261	5	6	2
1977	2.4	15	325	0	0	0	7	12.8	3 315	5	15	24
1978	40.0	223	2 921	1	10	0	6	10.2	3 101	1	4	0
1979	11.3	150	1 740	1	4	0	6	4.2	941	1	0	5
1980	57.0	86	5 274	1	60	0	7	18.1	4 686	4	25	64
1981	29.8	89	1 633	0	0	0	4	2.0	792	0	0	0
1982	7.0	122	1 450	0	0	0	8	5.0	1 944	2	4	5
1983	43.7	93	3 206	1	8	0	6	6.8	2 903	1	7	0
TOTAL	310.9	1529	29 241	10	104	8	88	112.1	1444	41	153	316

^a Stream treatments began in 1971 in Canada and 1972 in the United States. No streams were treated in 1972 and 1974 in Canada and the United States respectively.

^b Granules used during surveys in conjunction with treatments.

^c Granules used in treatments with TFM

4.0

METHODS OF ANALYSIS FOR TFM AND BAYER 73

Early methods for the detection of TFM and Bayer 73 lacked sensitivity and encountered frequent problems with interfering substances in samples. Current analytical systems for both TFM and Bayer 73 include gas-liquid chromatography, liquid scintillation spectroscopy and high performance liquid chromatography. TFM can also be quantitated using thin-layer chromatography, UV-VIS absorption spectroscopy and selected ion mass spectroscopy.

The commonly used methods used for TFM and Bayer 73 are listed in Tables 4-1 and 4-2 respectively.

4.1 QUANTITATIVE ANALYSIS FOR TFM AND BAYER 73 IN WATER DURING STREAM TREATMENTS

4.1.1 QUANTITATIVE ANALYSIS FOR TFM

The method initially developed to quantitate TFM in streams was a colorimetric technique based on the yellow color of the TFM molecule under alkaline (buffered) conditions (Smith *et al.* 1960). The problem of interfering substances was overcome either by preparing standards in stream water or by making corrections for background color. The method provides analytical accuracy to 0.1 mg.L^{-1} TFM and is still used to monitor TFM by the sea lamprey control units in Canada. One drawback to this technique however, is that it cannot be used to analyze Bayer 73.

4.1.2 QUANTITATIVE ANALYSIS FOR BAYER 73

Early methods for the colorimetric analysis of Bayer 73 in water lacked sensitivity, suffered from interferences and were too time consuming for field use (Strufe 1962; Daniels *et al.* 1965; El-Dib and Aly 1972). Dawson *et al.* (1978) developed a colorimetric technique suitable for use during stream treatments using either an acid or base hydrolysis. Bayer 73 was extracted from acidified water samples with chloroform and hydrolyzed to 2-chloro-4-nitroaniline (CNA) with either an acid or base. The CNA was then diazotized with sodium nitrite and an azo dye formed with either N-(1-naphthyl) ethylenediamine dihydrochloride (acid hydrolysis) or 1-naphthol (base hydrolysis). This method was tested during an actual field treatment and was found to be free from interference from TFM, phytoplankton or suspended particulate matter. However, tannins and lignins in the stream water apparently caused some interference. The method is sensitive to 0.005 mg.L^{-1} .

Luhning *et al.* (1979) developed a method to measure Bayer 73 based on gas liquid chromatography. Extracted Bayer 73 was quantitatively hydrolyzed with alkali to 2-chloro-4-nitroaniline (CNA),

Table 4-1. Methods of analysis used for TFM.

Analysis/Detector	Extraction	Derivative	Medium	Sensitivity	Reference
Colorimetric	none	none	water	0.1 mg.L ⁻¹	Smith <u>et al.</u> (1960)
Colorimetric	Dowex 1X8 resin	none	"	0.01 mg.L ⁻¹	Billy <u>et al.</u> (1965)
GLC/ ⁶³ Ni electron capture detector	XAD-7 resin	acetate	"	0.01 µg.L ⁻¹	Coburn and Chau (1976)
GLC/flame ionization detector	solvent extraction ethyl ether	methyl ether	"	-	Liu and Fox (1979)
GLC/tritium foil detector	solvent extraction benzene/hexane (1/1)	silyl ether	"	-	Thingvold and Lee (1981)
Capillary GLC/ ⁶³ Ni electron capture detector	solvent extraction ethyl ether	methyl ether	"	0.3 ng.L ⁻¹	Carey <u>et al.</u> (1982)
HPLC/ultraviolet	Sep Pak C ₁₈ column	none	"	0.005 mg.L ⁻¹	Dawson (1982)
GLC/ ⁶³ Ni electron capture detector	solvent extraction hexane-ethyl ether	methyl ether	tissue	0.01 µg.g ⁻¹	Allen and Sills (1974)

Table 4-2. Methods of analysis used for Bayer 73.

Analysis/Detector	Extraction	Derivative	Medium	Sensitivity	Reference
Colorimetric	chloroform	CNA (2-chloro-4-nitro-aniline)	water	0.005 mg.L ⁻¹	Dawson et al. (1978)
GLC/ ⁶³ Ni electron capture detector	acidic acetone	CNA (2-chloro-4-nitro-aniline)	water tissue sediment	1 µg.L ⁻¹ 0.01 µg.g ⁻¹ 0.01 µg.g ⁻¹	Luhning et al. (1979)
HPLC/ultraviolet	Sep Pak C ₁₈ column	none	water	0.005 mg.L ⁻¹	Dawson (1982)

which was subsequently partitioned into a solvent and analyzed by GLC. The sensitivity is 0.001 mg.L^{-1} .

4.1.3 SIMULTANEOUS ANALYSIS FOR TFM AND BAYER 73

Recently, Dawson (1982) developed a method to determine the concentrations of TFM and Bayer 73 simultaneously in stream treatments, using high-performance liquid chromatography. Previously, levels of TFM and Bayer 73 had to be analyzed separately. This method avoids interferences encountered in colorimetric techniques and eliminates the time consuming hydrolysis or derivatization reactions required for gas-liquid chromatography in the laboratory. Analyses for both chemicals can be completed in 10 minutes with a sensitivity of $< 0.005 \text{ mg.L}^{-1}$.

The method involves the addition of a combined buffer (pH 4) and internal standard (3-chlorodiphenylamine) reagent to the water sample, which is then injected through a Sep Pak C_{18} disposable cartridge which adsorbs and retains both the lampricides and the internal standard. A small volume of methanol quantitatively elutes the three chemicals from the cartridge, concentrates the sample and provides sample cleanup. The methanol extract can then be analyzed directly by HPLC with UV detection. Because the levels of Bayer 73 used in lampricide treatments are much lower than those of TFM, they must be concentrated, which can cause TFM chromatograms to go off-scale. However the use of a microprocessor data system quantifies off-scale peaks and yields results directly in units of concentration (mg.L^{-1}).

4.2 QUANTITATIVE ANALYSIS FOR TFM AND BAYER 73 IN WATER, SEDIMENTS AND TISSUES IN THE LABORATORY

4.2.1 QUANTITATIVE ANALYSIS FOR TFM IN WATER

Refinements of the method of Smith *et al.* (1960), through the use of anion exchange resins to concentrate the TFM, extended the detection limits below 0.1 mg.L^{-1} while simultaneously reducing interferences from TFM impurities and naturally occurring colored substances (Daniels *et al.* 1965; Billy *et al.* 1965).

Coburn and Chau (1976) developed a more sensitive method for analyzing TFM in natural waters using gas-liquid chromatography. They extracted TFM from acidified water samples on macroreticular resin XAD-7, eluted it from the column with ethyl ether and dried the extract before concentrating and partitioning it with potassium carbonate. TFM was then acetylated in an alkaline solution and the acetate derivative was extracted with benzene for analysis by electron capture detection. The limit of detection was $0.01 \text{ } \mu\text{g.L}^{-1}$.

More recently, with the development of commercially available capillary columns for GLC, sensitivity and selectivity have been

extended still further. Carey et al. (1982) reported that with this technique and using either an electron capture detector or a mass spectrometer monitoring m/e 191, as little as 1 pg of TFM (as the methyl ether) can be detected. This corresponds to a water concentration of 0.3 ng.L^{-1} (assuming extraction of a 4L water sample, preconcentration of the extract to about 1 mL and an injection of 1 μL).

4.2.2 QUANTITATIVE ANALYSIS FOR TFM RESIDUES IN TISSUES

For the analysis of TFM residues in biological tissues, Daniels et al. (1965) used a colorimetric method in which macerated fish tissue was hydrolyzed in hot 3N hydrochloric acid. Background color of the hydrolysate was reduced by one of three methods: (1) serial extractions with ether, methylene chloride and benzene; (2) cation exchange followed by methylene chloride extraction; and (3) ether extraction followed by anion exchange and desorption with amyl acetate-acetic acid. Billy et al. (1965) employed a modified version of this method to analyze stream fish exposed to TFM during treatment in the field and in laboratory uptake studies.

A gas-liquid chromatography method developed by Allen and Sills (1974) is free from the interferences encountered in earlier colorimetric techniques. Most analytical procedures for residues of TFM in tissues use this method or a modification of it. Fish muscle tissue was homogenized and extracted with hexane-ethyl ether. The extract was cleaned up by partitioning into 0.1N NaOH, acidifying the aqueous solution and partitioning again with hexane-ethyl ether. Following methylation with diazomethane, the methyl ether derivative was analyzed by GLC. Sensitivity of the method is better than $0.01 \text{ }\mu\text{g.g}^{-1}$.

Other techniques used in laboratory studies to measure the uptake, distribution, metabolism and elimination of TFM include thin-layer chromatography, radiolabelled TFM followed by liquid scintillation counting, infrared spectroscopy, UV spectroscopy and mass spectroscopy (Lech 1973b).

4.2.3 QUANTITATIVE ANALYSIS FOR BAYER 73 RESIDUES

Since the procedure of Dawson et al. (1978) is not sensitive enough to quantitate residues in fish, aquatic invertebrates or mud, the method of Luhning et al. (1979) (see Section 4.1.2) can be used. The sensitivity for analyzing Bayer 73 in samples of fish, invertebrates and mud is $0.01 \text{ }\mu\text{g.g}^{-1}$. Radiolabelled Bayer 73 has also been employed to study its uptake, distribution and elimination by fish in the laboratory (Schultz and Harman 1978b).

Churchill and Ku (1980) developed a method that uses liquid-liquid phase-transfer alkylation to form the N, O-dimethyl

derivative of Bayer 2353. The derivative and a similar internal standard are then compared by gas chromatography using an electron-capture detection method. The minimum detection limit in water is $10 \mu\text{g.L}^{-1}$. This method can also be used to determine residues in sediments and fish.

Muir and Grift (1980) reported the analysis of Bayer 2353 in water and sediment by gas chromatography of the methylated derivative. Using an electron capture detector, a water concentration of $16 \mu\text{g.L}^{-1}$ was readily determined. Detection limits were not stated but appeared to be $1 \mu\text{g.L}^{-1}$ in water and $0.1 \mu\text{g.g}^{-1}$ in sediment.

5.0 FATE OF TFM AND BAYER 73 IN THE AQUATIC ENVIRONMENT

5.1 ABIOTIC PROCESSES

5.1.1 VOLATILIZATION

TFM is stable in the aquatic environment and is not subject to volatilization (Kempe 1973; Thingvold 1975). Kempe (1973) found that aqueous solutions of TFM subjected to boiling in distilled water and trypticase soy broth for 45 minutes remained stable. Moreover, volatilization did not occur when open tubes of TFM solutions at pH's of 5.0, 7.0 and 10.0 were autoclaved for 2 hours. No information is available concerning the volatilization of Bayer 73.

5.1.2 HYDROLYSIS

Kempe (1973) established that TFM was not subject to hydrolysis since TFM solutions in open tubes at pH's of 5.0, 7.0 and 10.0 did not degrade after undergoing 2 hours of autoclaving.

According to Schultz and Harman (1978a), Bayer 73 is also not subject to hydrolysis. No degradation of ^{14}C -Bayer 2353 (Bayer 73 is the 2-aminoethanol salt of Bayer 2353) was observed in either distilled water at pH's of 5.0, 6.9 and 8.7 or pond water at pH 7.8 after 56 days in the dark. These results are in agreement with those of El-Dib and Aly (1976) who studied the base hydrolysis of Bayer 2353. They found that the half-life of this compound in 0.5N NaOH at 20°C was 19 days and concluded that hydrolysis would be a minor degradation pathway in surface waters (pH 6 to 9).

5.1.3 SEDIMENT BINDING

5.1.3.1 TFM

In sediment-water systems abiotic binding of TFM to the sediments has been demonstrated to various degrees (Bothwell *et al.* 1973; Kempe 1973; Thingvold 1975; NFRL 1983a, b, c) and was thought to be related to the organic content of the sediment itself (Kempe 1973; NFRL 1983a, b, c). Kempe (1973) observed that as the organic content of the sediment increased, the removal of TFM from the water also increased and, apparently, so did the adsorption to the sediments. In more detailed studies, the adsorption and desorption of 1 mg.L⁻¹ solutions of ^{14}C -TFM from 3 different sediment-types at a variety of pHs and temperatures was investigated (NFRL 1983a, b, c). The solutions (20 mL) and sediments (2 g dry weight) were allowed to equilibrate (>16 h) on an orbital shaker. Adsorption isotherms indicated that the adsorption maxima were not exceeded. Unfortunately the organic carbon content of each sediment type was not measured, so that soil sorption coefficients (K_{oc}) could not be calculated.

The results indicated that the amount of TFM adsorbed progressively decreased: (1) as the sediment type changed from predominately silt (Cedar River), to a sand/silt mixture (Ford River) to sand (Tahquamenon River); and (2) as the pH increased from 6 to 9 with each type of sediment. For example, at pH 7 the adsorption of TFM on silt sediments was about $3.5 \mu\text{g.g}^{-1}$ as opposed to only $0.3 \mu\text{g.g}^{-1}$ on sand. As the pH rose from 6 to 9, the amount of TFM adsorbed to silt decreased from $5.8 \mu\text{g.g}^{-1}$ to $1.1 \mu\text{g.g}^{-1}$.

Silt was the only sediment where the adsorption of TFM was influenced by temperature. The adsorption at pH 7 was slightly more at 5°C ($5.5 \mu\text{g.g}^{-1}$) than at 20°C ($3.7 \mu\text{g.g}^{-1}$). No data were available for the sand/silt mixture. The minimal adsorption of TFM on sand ($<1 \mu\text{g.g}^{-1}$) was identical at 5°C and 20°C .

In desorption experiments over 24 hours at pH 7.0, the amount of TFM released was measured as a percentage of the initial amount adsorbed and was found to be a function of sediment type and temperature. For instance, at 20°C there was a 100% release of TFM from sand but only a 65% release from silt. At 5°C the desorption from sand was halved while the release from silt declined to 35%. The influence of temperature was less pronounced on the sand/silt mixture from which roughly 70% of the TFM was desorbed at 20°C , whereas only 55% was released at 5°C .

5.1.3.2 RTFM

RTFM is 3-trifluoromethyl-4-aminophenol, a reduced form of TFM. Thingvold (1975) speculated that the observed abiotic binding of TFM to the sediments was accomplished by the reduction of the NO_2 group to an NH_2 group, which he felt would facilitate binding to other organics in the sediment through the amino group.

Studies at the National Fishery Research Laboratory (1983a, b, c) under the same conditions as for TFM, showed that ^{14}C -RTFM was consistently adsorbed to a greater extent than TFM, regardless of pH or sediment type. In addition, pH had no effect on the adsorption of RTFM although the sediment type did. For silt sediment, the quantity of RTFM adsorbed fluctuated somewhat between $5.5 \mu\text{g.g}^{-1}$ and $7.0 \mu\text{g.g}^{-1}$ from pH 6 to 9, whereas for TFM the adsorption progressively declined from $5.8 \mu\text{g.g}^{-1}$ to $1.1 \mu\text{g.g}^{-1}$. The same pattern held for sand/silt and sand mixtures. The adsorption of RTFM to sand sediment was lower than that for silt or sand/silt mixtures, ranging from 2.0 to $3.0 \mu\text{g.g}^{-1}$ over the pH range 6 to 9. There was no significant difference between adsorption at 5°C or 20°C at pH 7.0, for all types of sediments.

RTFM was adsorbed strongly to all sediments; during a 24-h period, less than 20% of that adsorbed was released at pH 7.0. Desorption was not significantly affected by temperature.

5.1.3.3 Bayer 73

Using the same procedures as those outlined for TFM (Section 5.1.3.1), it was found that the adsorption of Bayer 73 to river sediments was affected largely by the sediment type and to a lesser extent by pH and temperature (NFRL 1983a, b, c). At pH 7.0 and 20°C, the adsorption was greatest on silt ($9.3 \mu\text{g.g}^{-1}$), intermediate on sand/silt mixtures ($8.8 \mu\text{g.g}^{-1}$) and least on sand ($5.0 \mu\text{g.g}^{-1}$). Bayer 73 was adsorbed to a greater extent than either TFM or RTFM. Its adsorption was similar to that of TFM in that it was adsorbed most on silt, to an intermediate level on sand/silt and least on sand. Organic content may influence adsorption rates as well as particle size (Meredith 1971b). The lowest pH tested was 6.5 because of the low solubility and apparent precipitation of Bayer 73 at lower pH's.

For silt sediments, pH appeared to have no effect on adsorption, as the levels fluctuated from 2.8 to $4.0 \mu\text{g.g}^{-1}$ over the range 6.5 to 9.0. For the sand/silt mixture, the adsorption of Bayer 73 remained relatively stable ($8.0 - 8.8 \mu\text{g.g}^{-1}$) from pH 6.5 to 8.0, but dropped to $5.1 \mu\text{g.g}^{-1}$ at pH 9.0. The adsorption on sand showed a distinct decline with increasing pH, from $5.4 \mu\text{g.g}^{-1}$ at pH 6.5 to $0.9 \mu\text{g.g}^{-1}$ at pH 9.0.

Temperature had virtually no effect on the adsorption of Bayer 73, regardless of sediment type. For instance, at pH 7.0 the adsorption was almost identical at both 5°C and 20°C.

The comparative desorption of Bayer 73 from sediments was evaluated during a 24-hour period by replacing the overlying water several times. Roughly 65% of the initial amount adsorbed was released from silt sediments. In contrast, desorption from sand and sand/silt mixtures was only 25% and 15% respectively. Similarly, Muir and Yarechewski (1982) found very little desorption ($\leq 2\%$) of ^{14}C -Bayer 2353 from five different sediment samples. Temperature (5° and 20°C) had no effect on desorption regardless of sediment type.

The relatively strong adsorption of Bayer 73 by sediments, especially those from the Cedar River, may help explain the loss of Bayer 73 concentrations encountered during the treatment of some river systems. On the other hand, Muir and Yarechewski (1982) predicted that despite the strong adsorption of ^{14}C -Bayer 2353 to sediment, greater than 99% would be in solution, even in river water containing as much as 50 mg.L^{-1} suspended solids. They concluded that adsorption to suspended solids was unlikely to play a major role in the environmental fate of Bayer 2353 under field conditions. They did suggest that the rapid degradation of Bayer 2353 could be a contributing factor in reducing its efficacy following an application (see Section 5.2.1.2).

5.1.4 PHOTODECOMPOSITION

5.1.4.1 TFM

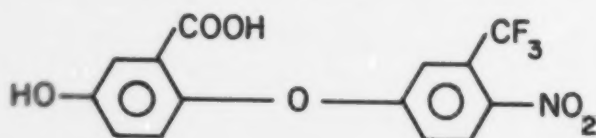
Recent studies have addressed the photodecomposition of TFM. The results of an initial study by Dawson (1973) were inconclusive. Aqueous solutions of TFM did not degrade in the presence of sunlight or a 275W ultraviolet lamp ($18.2 \mu\text{W} \cdot \text{cm}^{-1} \times 100$). Yet solutions of ^{14}C -TFM exposed to sunlight for 32 hours ($8 \text{ hours} \cdot \text{day}^{-1}$ for 4 days) displayed a 20% loss in activity and were estimated to have a half-life of about 100 hours. When TFM was spotted on silica gel thin-layer chromatography plates and exposed to either sunlight or UV light for 4 hours, 5 photoproducts became evident, one of which was thought to be RTFM.

Recently, Carey and Fox (1981) examined the photolysis of TFM in two Lake Ontario tributaries and in distilled water by irradiating the solution using either natural sunlight or UV light. They found that a $4.66 \text{ mg} \cdot \text{L}^{-1}$ concentration of TFM exposed to September sunlight was photodegraded, although the rate of disappearance was significantly lower in distilled water than in the natural waters. At an accumulated incident solar radiation of $5 \text{ kJ} \cdot \text{cm}^{-2}$, 60% of the TFM initially present had been photodegraded in both natural waters whereas only 50% had undergone photolysis in distilled water. This difference was related to pH. In distilled water the pH decreased to 5 during the photolysis reaction whereas in the streams, it remained at 7.8. Acidic solutions of TFM absorb less sunlight than basic solutions at the same concentration. Hence, under conditions of less than total absorbance, the photolysis rate in the distilled water decreases as the pH drops (Carey and Fox 1981).

The half-life of TFM in flasks of stream water exposed to sunlight was estimated at 3 days, based on the average daily solar illumination for September 1979. Using the disappearance quantum yields at wavelengths of 300 and 360 nm and assuming a photoactive range of 280 to 450 nm and a stream depth of 55 cm, Carey and Fox (1981) predicted that the half-life of TFM in streams would be 3.5 days in spring, 3 days in summer and 5 days in autumn. These estimates assume sunny weather; the actual half-lives would be longer under overcast conditions and/or in streams that are heavily shaded or greater than 55 cm in depth.

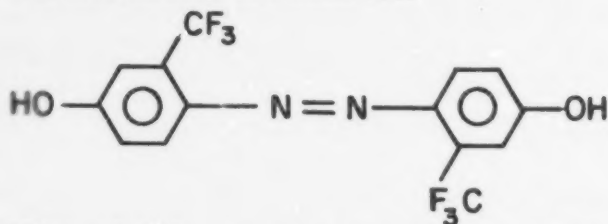
Recently, Scott *et al.* (1984) observed that the disappearance of TFM added to three lined artificial ponds was a first order process. The disappearance rate constants and half-lives were 0.078 ± 0.006 ($t_{1/2} = 9$ days) for purified TFM and 0.060 ± 0.004 ($t_{1/2} = 12$ days) for the commercial formulation. None of the known degradation products was observed. The photochemical half-life predicted following the data and calculations of Carey and Fox (1981) was 9 days, once allowance was made for the mean depth of 1.25 m. The difference in rates observed between purified and formulated TFM was thought to be due to the presence of light-absorbing material in the formulation.

Two major and five minor photoproducts were determined by capillary gas chromatography, and mass spectra were obtained for all but one of the minor products. In contrast to the results of Dawson (1973), RTFM was not detected. One of the major products was shown to be 2,5-dihydroxybenzoic acid while the other was thought to be 2-(3-trifluoromethyl-4-nitrophenoxy)-5-hydroxybenzoic acid:



The latter was the only photoproduct not accounted for in the photolysis pattern illustrated in Figure 5-1.

Fox and Carey (1984) examined streams undergoing TFM treatment for the presence of the predicted photoproducts. During the 1981 treatment of Lynde Creek, Ontario, all of the major photoproducts were observed at the outlet of this stream (F, D, B, C from Figure 5-1 as well as 2-(3-trifluoromethyl-4-nitrophenoxy)-5-hydroxybenzoic acid), but were absent from the treatment site. In addition, a new compound, whose mass spectrum is similar to that expected for 2,2'-bis-trifluoromethyl-5,5'-dihydroxyazobenzene, was observed.



The route of formation of this compound is unknown at the present time.

5.1.4.2 Bayer 73

A single laboratory study is available concerning the photodegradation of Bayer 73. Shultz and Harman (1978a) investigated the photolysis of ^{14}C -Bayer 2353 on TLC plates, glass slides and in distilled water after exposure to long wave (290-405 nm) UV irradiation. When ^{14}C -Bayer 2353 was spotted and irradiated on TLC silica gel plates, 95% was degraded into 7 unidentified photoproducts after 168 hours of exposure. Similar results were observed for ^{14}C -Bayer 2353 on glass slides, except that the degradation occurred more rapidly during the initial 24 hour period.

Exposure of ^{14}C -Bayer 2353 in distilled water resulted in a much slower rate of degradation. For example, it required 14 days for 95% of the initial 5 μg of ^{14}C -Bayer 2353 to be photolyzed in 100 mL of

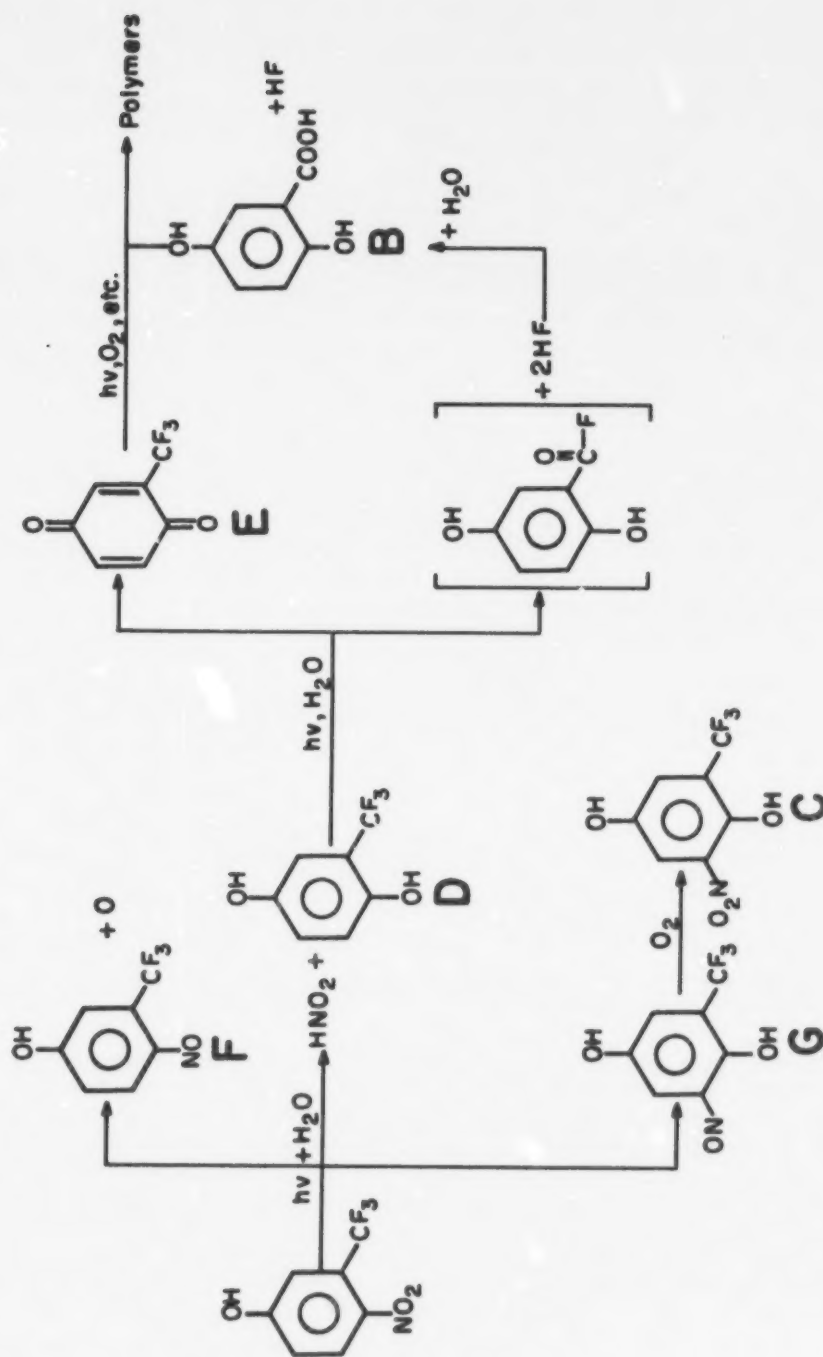


Figure 5-1. Proposed route of photolysis of TFM (from Carey and Fox 1981).

distilled water. Four photoproducts were found, none of which was identified.

5.2 BIOTIC PROCESSES

5.2.1 MICROBIAL TRANSFORMATION OF TFM AND BAYER 73

5.2.1.1 TFM

The transformation of TFM by microorganisms has been investigated in aerobic and anaerobic systems in the laboratory using both water and sediment and sediment-free systems. The water and sediment technique involved the incubation of sediments containing microorganisms, obtained from lakes and rivers, with water that held concentrations of TFM similar to those used in treating lamprey-infested tributaries. In sediment-free systems, microorganisms obtained from the interstitial water of sediments from various lakes and rivers were added to TFM solutions. In both cases, the amount of TFM and its derivatives were analyzed at selected time intervals.

Although some of the results tend to conflict, it appears that the type of sediment (see Section 5.1.3.1) and the presence or absence of oxygen can influence the fate of TFM. In aerobic environments, microorganisms had no effect on TFM in either water and sediment (Bothwell *et al.* 1973; Thingvold 1975) or sediment-free systems (Liu and Fox 1979; Thingvold and Lee 1981). Bothwell *et al.* (1973) carried out all of their experiments in the dark to preclude the possibility of photochemical degradation; they used ^{14}C -labelled TFM together with thin layer chromatography and gas chromatography (GC) to identify TFM and its derivatives. The concentration of TFM in the water, had not changed significantly after 2.5 months. Similarly, in sediment-free systems, TFM remained unaltered over periods of 36 to 80 days under a variety of conditions (Liu and Fox 1979; Thingvold and Lee 1981). Microorganisms did not transform TFM regardless of whether it was the sole carbon source or whether auxiliary carbon sources were added (Liu and Fox 1979; Thingvold and Lee 1981). Neither the use of previously acclimated microorganisms nor an increase in the surface area for colonization had any effect on the transformation of TFM (Thingvold and Lee 1981).

Contrary to the work of Thingvold (1975) and Thingvold and Lee (1981), it appears that under anaerobic conditions TFM can be reduced to 3-trifluoromethyl-4-aminophenol (RTFM) in water and sediment (Bothwell *et al.* 1973) and sediment-free experiments (Liu and Fox 1979). Bothwell *et al.* (1973) determined that the time required for a 100% microbial reduction of TFM varied from 5 to 20 days. The process could be accelerated if supplemented by an auxiliary source of carbon (Liu and Fox 1979).

Thingvold (1975) suggested that TFM was not subject to microbial action since autoclaving the sediment or adding bacteriostatic

agents had no effect on the rate or extent of removal of TFM from the water. He speculated that the sorption of TFM to the sediments, which was accelerated under anaerobic conditions, was the result of the NO_2 group being converted to an NH_2 group (RTFM), thereby facilitating binding to organic material in the sediment. Similarly, Thingvold and Lee (1981) detected no microbial activity and no alteration in the form of TFM in sediment-free experiments. Although the results under anaerobic conditions observed by Bothwell et al. (1973) and Liu and Fox (1979) seem to conflict with those of Thingvold (1975) and Thingvold and Lee (1981), it is important to note that TFM was not completely degraded in any of these studies.

Kempe (1973) did not indicate whether his aquarium system was aerobic or anaerobic, but did report that TFM was eliminated from the water and sediment after 34 days. He attributed the loss to microbial action. However, the only measurement technique used was a colorimetric procedure for TFM, so it was not possible to identify any products. Furthermore, the method may not have been reliable since the unexplained darkening of test vessels by microorganisms has occurred in other TFM biotransformation studies (Christianson 1977). Bothwell et al. (1973) speculated that what Kempe (1973) had observed was actually the reduction of TFM to RTFM under anaerobic conditions.

Based on studies on microbial transformation, it appears that TFM remains unaltered in aerobic environments whereas in anaerobic environments it is reduced to RTFM. This difference may be related to the nature of the microorganisms present. Since TFM is a powerful uncoupler of oxidative phosphorylation in sediment microorganisms (Liu et al. 1977), it would inhibit aerobic microbial attack. However, anaerobic microorganisms do not utilize oxidative phosphorylation to obtain energy and so would not be inhibited by TFM.

5.2.1.2 Bayer 73

The transformation of Bayer 2353 has been investigated in sediment-water systems (Muir and Yarechewski 1982). Radiolabelled Bayer 2353 was added to water at a concentration of 1 mg.L^{-1} and incubated with pond and river sediments for 128 days at 25°C under static conditions. The radioactivity (less than 33% of which was in the water) decreased rapidly in both the water and sediments; the half-lives in the water and river sediments were 0.83 and 3.9 days respectively. Microorganisms were thought to have been responsible for the observed disappearance because in autoclaved samples, the transformation was either limited (pond sediment) or absent altogether (river sediment). Some bacteria are able to use Bayer 73 as a nitrogen source (Etges et al. 1965, 1969; Bell et al. 1966). Two genera, Pseudomonas sp. and Aerobacter sp., are found in the water and sediment in the Caribbean area as well as in the intestinal tract of the snail (Biomphalaria glabrata). Both bacteria have been identified as being capable of

transforming Bayer 73 (Bell et al. 1966; Etges et al. 1969). However, it should be pointed out that in the Great Lakes, water temperatures are much lower and hence the rate of transformation would probably be slower.

One major transformation product in both the water and sediments was found, 2',5-dichloro-4'-aminosalicylanilide (Muir and Yarechewski 1982). Its formation, which represented over 50% of the radioactivity extracted from the sediments, was favored under anaerobic conditions at low redox potential. The rate of transformation was much slower than that of Bayer 2353; the half-life in river sediment was 143 days.

Another transformation product, 2-chloro-4-nitroaniline (CNA) was detected, suggesting that, contrary to Schultz and Harman (1978a), hydrolysis had occurred. However, the salicylic acid moiety was not observed, indicating that it was either transformed rapidly, sorbed to the sediments or that degradation of that end of the molecule had occurred prior to hydrolysis.

5.2.2 BIOLOGICAL CYCLING

The biological cycling of TFM and Bayer 73 refers to the uptake, distribution, metabolism and elimination of these compounds by exposed aquatic organisms. The terms bioconcentration, bioaccumulation and biomagnification have been used inconsistently in the literature to describe the uptake of these substances. For the purposes of this discussion, the definitions of Brungs and Mount (1978) will be used. Bioconcentration is the process whereby chemical substances enter aquatic organisms through the gills or other epithelial tissue, directly from the water. Bioaccumulation is a broader term which includes bioconcentration plus any uptake of chemical residues from dietary sources. Biomagnification refers to the process by which the tissue concentration of bioaccumulated chemical residues increases as materials pass up the food chain through two or more trophic levels. In the studies of the effects of TFM and Bayer 73 on aquatic organisms reviewed herein, the only sources have been ambient i.e. uptake from water. Thus, as defined there are no data concerning bioaccumulation or biomagnification.

5.2.2.1 Uptake, Distribution and Elimination of TFM

Aquatic Macrophytes, Algae and Benthic Invertebrates

The bioconcentration of TFM by algae, aquatic macrophytes (Maki and Johnson 1977b) and invertebrates (Kawatski and Bittner 1975; Sanders and Walsh 1975; Maki and Johnson 1977b) follows a biphasic pattern. An initial rapid uptake is followed by a more gradual accumulation until a plateau is reached.

The whole-body levels accumulated by the various components of a model stream community exposed to an initial concentration of 9 mg.L^{-1} over a 24-h period are given in Table 5-1. Although the residues taken up differ among species, the accumulations, as reflected by their bioconcentration factors (BCF), are all relatively low, i.e. < 100 (Stern and Walker 1978). However, it should be pointed out that because of the experimental design of the study, the bioconcentration factors were probably underestimated. For example, the organisms were not exposed in a flow-through system, and hence, were not exposed to a constant concentration of 9.0 mg.L^{-1} TFM (the concentration declined from 9.0 to 6.9 mg.L^{-1} after 24 h). In addition, it was not determined if the tissue levels had reached an equilibrium. Despite these problems, it should be noted that annelid worms exhibited one of the highest accumulations of TFM ($454.8 \text{ } \mu\text{g.g}^{-1} \text{ d.w.}$) of the organisms tested. They appear to have a pronounced ability to concentrate phenolic contaminants. For instance, Metcalfe *et al.* (1984) observed that leeches from a creek receiving leachates from an abandoned chemical waste dump, contained levels of chlorophenols 1 to 2 orders of magnitude greater than those found in 13 other benthic invertebrates. In the same study, aquatic oligochaetes had comparable residues.

A general uptake trend shows that soft-bodied animals, e.g. annelids (BCF = 50.5) tend to concentrate higher levels of TFM than those with hard exoskeletons, e.g. crayfish (BCF = 1.2) and snail (BCF = 9.1), indicating that the body burden may possibly be related to the permeability of the test species' integument. Hansen *et al.* (1980) observed that about 60% of the TFM residues were found within the body wall and haemolymph of the aquatic midge, *Chironomus tentans*, after a 24-h sublethal exposure to 0.07 mg.L^{-1} TFM, suggesting that the integument was involved in uptake. The longer half-life of TFM in organisms inhabiting pools (e.g. the isopod *Asellus militaris* and annelids), may indicate continued uptake and exchange of TFM because of their close association with organic matter in the sediment (Maki and Johnson 1977b).

Transformation of TFM by invertebrates was studied by Kawatski and Bittner (1975). Using fourth instar larvae of the aquatic midge, *Chironomus tentans*, exposed to 0.224 mg.L^{-1} ^{14}C -TFM for 12 hours, followed by a 16-h recovery period, they found that approximately 45% of the accumulated ^{14}C -TFM residue was biotransformed into either TFM-glucuronide or galacturonide, a sulfated TFM derivative and an unidentified compound. In addition, about 2.5% of the TFM was reduced to RTFM. Nevertheless, the ability to biotransform TFM appeared to be limited, since over 50% of the residue remained as unaltered TFM.

Following the 24-hour exposure period, the biota listed in Table 5-1 were held in TFM-free water in a flowing system to determine their respective rates of clearance. The total residue levels were eliminated rapidly, except by annelid worms. In most organisms the majority of the body burden was cleared within the first day of transfer

Table 5-1. Accumulation, bioconcentration factor and half-life of TFM in various components of a model stream community exposed to a concentration of 9 mg.L⁻¹ TFM for 24 h (hardness = 211 mg.L⁻¹ CaCO₃, pH = 7.79) (adapted from Maki and Johnson 1977b).

Matrix	Accumulation ($\mu\text{g.g}^{-1}\text{.d.w.}$)	Bioconcentration Factor	Half-Life (h)
Green algae	61.0	6.8	65.2
<u>Cladophora</u> sp.			
<u>Stigeoclonium</u> sp.			
Green algae ^a	106.3	11.8	25.8
<u>Cladophora</u> sp.			
<u>Stigeoclonium</u> sp.			
Aquatic macrophytes			
<u>Ceratophyllum demersum</u>	109.3	12.1	437.5
<u>Elodea canadensis</u>	49.8	5.5	87.4
Annelid worms	454.8	50.5	5295
Isopod			
<u>Asellus militaris</u> ^b	151.2	16.8	194.4
Amphipod			
<u>Gammarus pseudolimnaeus</u>	169.8	18.9	26.2
Crayfish	11	1.2	7.2
<u>Orconectes propinquus</u>			
Mayfly			
<u>Hexagenia</u> sp.	76.9	8.5	38.6
Caddisfly ^a			
<u>Glossoma</u> sp.	306.6	34.1	7.9
<u>Limnephilus</u> sp.	174.3	19.4	14.0
<u>Brachycentrus americanus</u>	559.4	62.2	19.5
Snail			
<u>Physa</u> sp.	81.8	9.1	23.2
Fingernail clam	121.4	13.5	7.9
<u>Pisidium</u> sp.			
Sediment	35.2	3.9	171.7

^a From riffle area, all other species from pool area.

^b The exact identity of this organism cannot be determined.

to clean water, although detectable residues were present in most components 2 to 4 weeks after exposure. Similar results were observed for three different tissues in the freshwater mussel, Anodonta sp. (Maki and Johnson 1976a). On the other hand, a half-life of 5295 hours for TFM in annelids (Table 5-1) is suggestive of a very limited clearance capacity. Similarly, Metcalfe et al. (1984) determined that the leech Dina dubia was unable to eliminate even a portion of a body burden of chlorophenols during a 2-week period in clean water. It appears then that annelids may be incapable of metabolizing and/or excreting phenolic compounds.

Gilderhus et al. (1975) examined residues of TFM in algae, aquatic macrophytes and invertebrates after treatment of a stream with 11 mg.L⁻¹ TFM for 12 hours. Their results are similar to those of Maki and Johnson (1977b). The whole-body wet weight residues ranged from a low of 0.8 µg.g⁻¹ in crane fly larvae to a high of 21.4 µg.g⁻¹ in oligochaetes immediately after treatment. In the 96-h period following treatment, 93-99% of the TFM initially concentrated had been cleared by all species examined, except the annelids. They were apparently eliminated by the treatment since no individuals could be found afterwards.

In a laboratory study of the uptake of TFM by the aquatic midge, Chironomus tentans, Kawatski and Bittner (1975) noted that the elimination of residues was more complete if the recovery water was renewed. For example, after a 48-h recovery period following an 8-h exposure to 0.09 mg.L⁻¹ TFM, the residue levels with and without changes in recovery water were 10 µg.g⁻¹ and 20 µg.g⁻¹ respectively. This suggests that in streams where TFM is applied, the flowing water would assist in the elimination of TFM residues.

Since the TFM residues in algae, aquatic plants and invertebrates, other than annelids, are eliminated rapidly (within 24 to 96 h in most cases) there is little likelihood that biomagnification will occur (Gilderhus et al. 1975; Sanders and Walsh 1975; Maki and Johnson 1977b).

Fish

The uptake of TFM and distribution within different tissues of various species of fish are illustrated in Tables 5-2 and 5-3. Total TFM levels are low in the blood, brain (Table 5-2) and muscle (Table 5-3). Schultz et al. (1979) found that in fasted largemouth bass, levels of TFM in all of the tissues examined had reached equilibrium after the fish had been exposed for 12 hours to 1 mg.L⁻¹ of ¹⁴C-TFM. Concentrations of total TFM in the blood and brain were <2 µg.g⁻¹ (Table 5-2). Similarly, Sills and Allen (1975) showed that the level of TFM in the muscle tissue of a number of Great Lakes species was <1 µg.g⁻¹ wet weight following a 12-h exposure to 1 mg.L⁻¹ TFM (Table 5-3).

Table 5-2. Uptake and distribution of the various forms of TFM in bile and different tissues of fish.

Species	Bile/ Tissue	Exposure Time (h)	Concentration TFM in Water (mg.L ⁻¹)	Concentration TFM in Tissue (µg.g ⁻¹)		Total TFM	Reference
				Free TFM	Conjugated TFM		
Rainbow trout (<u>Salmo gairdneri</u>)	plasma	2	5.0	2.73	0.87	3.60	Hunn and ^α Allen (1975b)
		12	1.0	0.27	-	0.27	
	bile	2	5.0	4.12	197	201	"
		12	1.0	1.24	510	511	
Largemouth bass (<u>Micropterus</u> <u>salmoides</u>)	blood	12	1.0	-	-	1.71	Schultz ^β et al. (1979)
		24	1.0	-	-	1.29	
	bile	12	1.0	-	-	696.4	"
		24	1.0	-	-	1497.3	
	liver	12	1.0	-	-	22.52	"
		24	1.0	-	-	18.03	
	kidney	12	1.0	-	-	15.35	"
		24	1.0	-	-	13.04	
	brain	12	1.0	-	-	1.52	"
		24	1.0	-	-	1.46	

^α Water hardness = 238-258 mg.L⁻¹ CaCO₃, pH = 7.6-7.9, temperature = 12°C.

^β Water hardness = - , pH = 6.8, temperature = 12°C.

Table 5-3. TFM residue levels in the muscle tissue of 7 species of fish after exposure to 1 mg.L⁻¹ TFM for 12 h and during a 24-h period in "clean" water (from Sills and Allen 1975).

Species	Temp. (°C)	Water Hardness (mg.L ⁻¹ CaCO ₃)	pH	Withdrawal Interval (h)	Muscle Tissue Concentration (µg.g ⁻¹ wet weight)
Rainbow trout (<u>Salmo gairdneri</u>)	12	40-48	7.2-7.6	0	0.30
				12	0.01
				24	-
Brown trout (<u>Salmo trutta</u>)	12	40-48	7.2-7.6	0	0.77
				12	0.13
				24	0.10
Lake trout (<u>Salvelinus namaycush</u>)	12	40-48	7.2-7.6	0	0.11
				12	0.02
				24	< 0.01
White bass (<u>Morone chrysops</u>)	12	40-48	7.2-7.6	0	0.02
				12	< 0.01
				24	< 0.01
Largemouth bass (<u>Micropterus salmoides</u>)	18.5	20-22	6.5-6.9	0	0.32
				12	< 0.01
				24	< 0.01
	14	-	6.8	0	0.86 ^α
				12	ND ^β
				24	ND
Bluegill (<u>Lepomis macrochirus</u>)	18.5	20-22	6.5-6.9	0	0.21
				12	0.04
				24	0.01
Channel catfish (<u>Ictalurus punctatus</u>)	18.5	40-48	7.2-7.6	0	0.77
				12	0.01
				24	< 0.01

^α From Schultz et al. (1979).

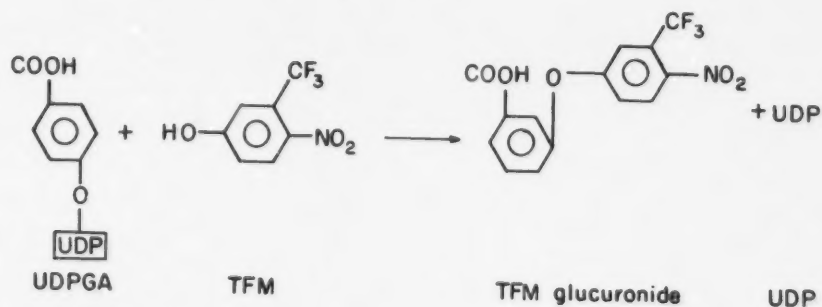
^β ND - Not detected.

In contrast, the concentrations of TFM in the liver and kidney of fasted largemouth bass were substantially higher at 22.52 and 15.35 $\mu\text{g.g}^{-1}$, respectively. TFM continued to accumulate in the bile after the exposure period, so that after 24 hours it had reached a concentration roughly 1500 times that of the exposure solution. In fasted rainbow trout, the bile also contained very high levels of TFM (511 $\mu\text{g.g}^{-1}$), virtually all of which was in the conjugated form (Table 5-2). In feeding fish, bile flow would significantly reduce buildup of TFM in the gallbladder.

The *in vivo* exposure of rainbow trout to TFM results in the uptake of TFM and conjugation with glucuronic acid, followed by excretion in the bile (Lech and Costrini 1972; Lech 1973b, 1974; Lech et al. 1973). Schultz et al. (1979) observed the same phenomenon in largemouth bass, *Micropterus salmoides*. *In vitro* studies of liver homogenates of rainbow trout revealed that the liver was a site of TFM conjugation (Lech and Costrini 1972; Kawatski and McDonald 1974). Tests with liver extracts from other species (white sucker, *Catostomus commersoni*, bluegill sunfish, *Lepomis macrochirus*, and largemouth bass) revealed that they were also able to conjugate TFM (Kawatski and McDonald 1974). TFM was also reduced to RTFM and another TFM metabolite by liver homogenates; however, only conjugation has been shown to occur *in vivo* (Lech and Costrini 1972; Kawatski and McDonald 1974).

Extracts of rainbow trout kidneys have also been shown to conjugate TFM (Lech and Costrini 1972), and conjugated TFM has been found in kidney tissue of TFM-exposed largemouth bass (Schultz et al. 1979). Hunn and Allen (1975a) observed that coho salmon, *Oncorhynchus kisutch*, excreted conjugated TFM in their urine. Overall, it appears that in fish, accumulated TFM is conjugated with glucuronic acid and is subject to biliary and renal excretion.

The conjugation or addition reaction occurs when glucuronic acid combines with TFM forming the corresponding glucuronide. The general process, as outlined by Goldstein et al. (1974) for mammals, is believed to be as follows: enzymes (transferases) in the soluble fraction of the liver catalyze the synthesis of uridine diphosphate - glucuronic acid (UDPGA), which in turn serves as a donor of glucuronic acid. The hydroxyl group in the TFM molecule is conjugated with glucuronic acid to form a glucuronide. The same pathway may also occur in fish.



In a long-term study, Dwyer et al. (1978) established that adult brook trout, Salvelinus fontinalis, exposed to 5.71 mg.L^{-1} for 137 days contained residues in the muscle, offal and eggs of 0.11, 0.38 and $2.8 \text{ } \mu\text{g.g}^{-1}$, respectively. These accumulations represent BCFs of < 1 in all cases. Considering the length of this exposure, it is unlikely that TFM would be concentrated to any great extent in fish during a normal stream treatment. Indeed, in another experiment simulating the use of TFM in the field, adult brook trout subjected to 5.71 and 6.43 mg.L^{-1} TFM for 12 hours contained no detectable TFM residues (Dwyer et al. 1978).

Data on the elimination of TFM and its derivatives from various tissues in fish are presented in Tables 5-3 and 5-4. In general, TFM is cleared from all tissues (blood, muscle, kidney, liver and brain), except bile, within 24 h. In contrast, the levels of TFM in the bile tend to increase during the first 8 h of recovery before falling after 24 h to about the same levels observed at the end of the exposure period (Table 5-4). TFM in the bile is almost entirely conjugated with glucuronic acid, again indicating that the liver is the primary site of conjugation and secretion of TFM into the gallbladder (Schultz et al. 1979). Studies to elucidate the ultimate fate of the conjugated TFM have not been carried out, but it has been suggested that some of the conjugated TFM is probably excreted in the feces while the remainder is likely resorbed and excreted by the kidney. Data in Table 5-4 does indicate that the kidney is involved in the excretion of TFM. Hunn and Allen (1975a) determined that TFM was present almost exclusively in the conjugated form in the urine of coho salmon during the 4-h exposure period, but that the concentration peaked at $61.55 \text{ } \mu\text{g.g}^{-1}$ after 4 h in TFM-free water. Thereafter the excretion of conjugated TFM declined rapidly so that only $0.26 \text{ } \mu\text{g.g}^{-1}$ was detected in the urine at 20 h postexposure. Over the entire study period, about 35 times more conjugated TFM was eliminated than free TFM. The fate of excreted TFM (either free or conjugated) has not been determined.

The lack of bioaccumulation of TFM from the water by fish in laboratory studies is supported by data from the field. Twelve hours after treatment of a stream with 11 mg.L^{-1} TFM, levels in rainbow trout and cyprinids ranged from 4.3 to $11.4 \text{ } \mu\text{g.g}^{-1}$ wet weight (Gilderhus et al. 1975). Within 96 h these residues had declined 99%. Furthermore, samples of lake trout and chinook salmon, Oncorhynchus tshawytscha, from the Upper Great Lakes did not contain detectable residues of TFM (limit of detection $< 0.01 \text{ } \mu\text{g.g}^{-1}$) (Sills and Allen 1976). Hence, the rapid elimination of TFM residues together with the absence of detectable levels of TFM in Great Lakes fish suggests that TFM is not subject to bioconcentration or biomagnification.

Sea Lampreys

The uptake of TFM by sea lampreys was studied by Lech and Statham (1975). For comparative purposes, the whole body content of

Table 5-8. Clearance of the various forms of TFM from different tissues and body fluids of fish.

Species	Tissue/ Body Fluid	Exposure Time (h)	Concentration in Water (mg.L ⁻¹)	Recovery Period (h)	Free TFM	Conjugated TFM	Concentration TFM in Tissue (µg.g ⁻¹)	Total TFM	Reference
Coho salmon (<i>Oncorhynchus kisutch</i>)	urine	4	3.1	-	1.39	19.94	21.33	Hunn and Allen ^a	
	-	-	0	4	0.68	61.55	62.23	(1975a)	
	-	-	0	8	0.43	7.87	8.3		
	-	-	0	20	0.07	0.26	0.33		
Rainbow trout (<i>Salmo gairdneri</i>)	plasma	-	0	20	-	-	< 0.1	"	
	bile	-	0	20	5	145	151	"	
	plasma	12	1.0	-	0.27	-	0.27	Hunn and Allen ^b	
	-	-	0	4	ND	-	-	(1975b)	
Largemouth bass (<i>Micropterus salmoides</i>)	-	-	0	8	ND	-	-		
	-	-	0	24	ND	-	-		
	bile	12	1.0	-	1.24	510	511.24	"	
	-	-	0	4	0.78	730	730.78		
Schultz et al. ^c	-	-	0	8	1.12	920	921.12		
	-	-	0	24	0.17	410	410.17		
	blood	12	1.0	-	-	-	1.70	Schultz et al. ^c	
	-	-	0	4	-	-	0.96	(1979)	
Schultz et al. ^c	-	-	0	8	-	-	0.33		
	-	-	0	24	-	-	0.14		
	bile	12	1.0	-	-	-	823.6	"	
	-	-	0	4	-	-	1263.1		
Schultz et al. ^c	-	-	0	8	-	-	1069.4		
	-	-	0	24	-	-	796.3		
	liver	12	1.0	-	-	-	14.22	"	
	-	-	0	4	-	-	4.21		
Schultz et al. ^c	-	-	0	8	-	-	2.21		
	-	-	0	24	-	-	2.35		
	kidney	12	1.0	-	-	-	17.38	"	
	-	-	-	4	-	-	6.62		
Schultz et al. ^c	-	-	-	8	-	-	3.31		
	-	-	-	24	-	-	0.54		
	brain	12	1.0	-	-	-	2.47	"	
	-	-	-	4	-	-	0.87		
Schultz et al. ^c	-	-	-	8	-	-	0.52		
	-	-	-	24	-	-	0.23		

^a Water hardness = 238-258 mg.L⁻¹ CaCO₃, pH = 7.6-7.9, temperature = 12°C.^b Water hardness = 238-258 mg.L⁻¹ CaCO₃, pH = 7.6-7.9, temperature = 12°C.^c Water hardness = 238-258 mg.L⁻¹ CaCO₃, pH = 7.6-7.9, temperature = 12°C.

free and conjugated TFM during a 4-h exposure to a sublethal concentration of 0.25 mg.L^{-1} ^{14}C -TFM is given in Figure 5-2 for both lamprey ammocoetes and rainbow trout. Two distinct results are evident. First, the total amount of TFM accumulated by sea lampreys is significantly greater than that by trout. Second, the amount of TFM conjugated by sea lampreys is only a fraction (0-25%) of that conjugated by trout. For instance, after 4 h, about $6.42 \text{ } \mu\text{g.g}^{-1}$ was concentrated by the lamprey larvae, of which only 7% was conjugated. In contrast, only $2.49 \text{ } \mu\text{g.g}^{-1}$ was accumulated by trout, and roughly 70% of the residue was conjugated. At the end of the experiment (4 h), it appeared that the uptake of total TFM had peaked in the ammocoetes whereas it was still increasing in the trout. Unfortunately, the experiment was not carried out for 8 to 20 hours, to simulate a field treatment, to establish whether this pattern of uptake would remain constant.

Unlike the laboratory study discussed above, concentrations of TFM applied in the treatment of streams to kill ammocoetes are considerably higher. The larvae become irritated initially and swim to the surface before sinking to the bottom and dying. The moribund larvae, containing residues of TFM, have been observed being consumed by sea gulls (Moffett 1958). Presumably other organisms feed on them as well, e.g. waterfowl, fish, crayfish, caddisfly larvae. Unfortunately, no data are available concerning the levels of TFM in freshly-killed ammocoetes. Information on the oral toxicity of TFM to birds and mammals is presented in Sections 13 and 14 respectively.

Amphibians, Reptiles, Birds and Mammals

No studies have been carried out on the accumulation, distribution or elimination of TFM by amphibians, reptiles or birds. With respect to mammals, the metabolism of TFM has been investigated in the rat (Lech 1971). TFM injected into the rat was excreted in the urine as RTFM and as polar derivatives of both TFM and RTFM. Some of the polar derivatives appeared to be glucuronide conjugates. A quantitative experiment using ^3H -TFM, showed that approximately 60% of the initial injected dose of 12 mg.kg^{-1} was excreted in the urine within 24 h and that only 6% was in the form of unaltered TFM.

5.2.2.2 Uptake, Distribution and Elimination of Bayer 73

Invertebrates

Aquatic insect larvae and crustaceans exposed to ^{14}C -Bayer 2353 in a flow-through system over a 5-day period tended to accumulate levels quickly, so that equilibrium concentrations were generally reached within 24 h (Sanders 1977). For example, the whole body residue of ^{14}C -Bayer 2353 in the scud after a 24-h exposure to 0.0012 mg.L^{-1} was $0.080 \text{ } \mu\text{g.g}^{-1}$ wet weight; after 120 h it was only $0.085 \text{ } \mu\text{g.g}^{-1}$ wet

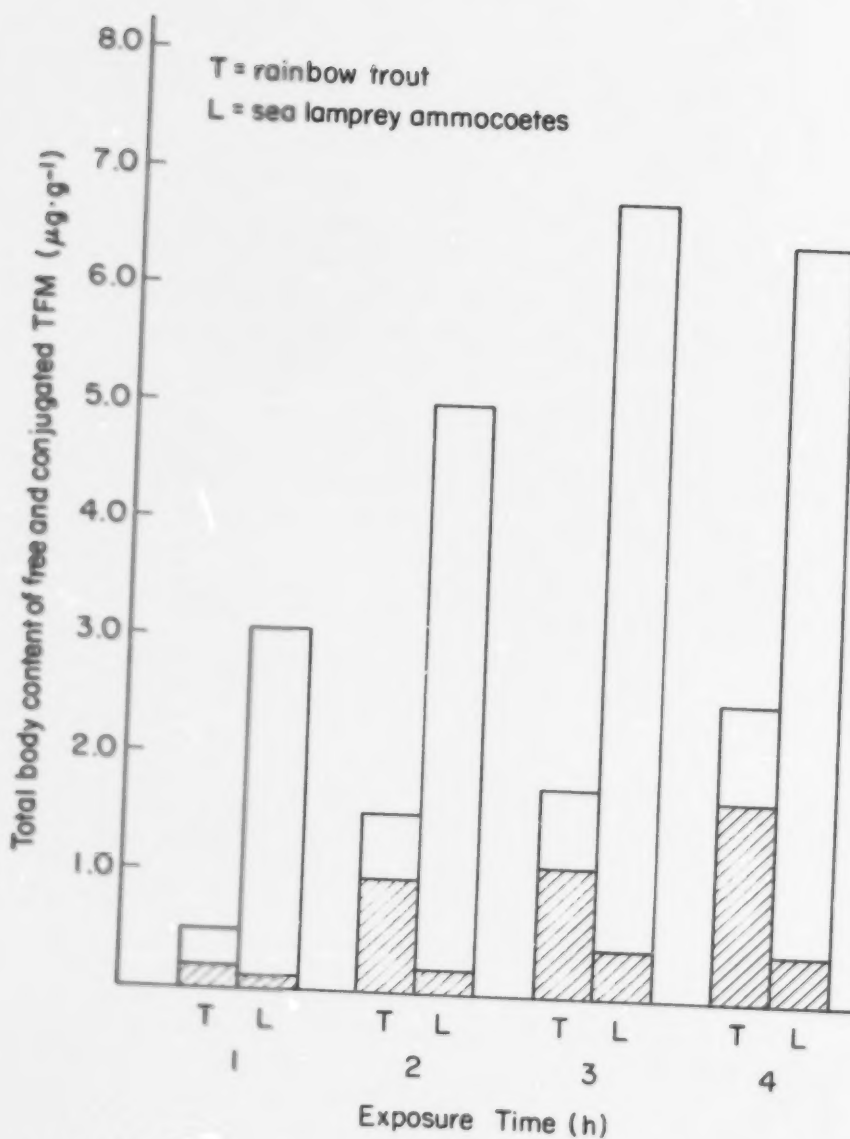


Figure 5-2. Total body content of free TFM and TFM glucuronide in rainbow trout and sea lamprey ammocoetes exposed to $0.25 \text{ mg}\cdot\text{L}^{-1}$ ^{14}C -TFM (adapted from Lech and Statham 1975).

 = total TFM (free TFM + TFM glucuronide)

 = TFM glucuronide

weight. The data in Table 5-5 suggest that the residue levels were not significant, because for all species tested the BCFs were < 100 (Stern and Walker 1978). As was the case for TFM, the uptake appeared to be greater for soft-bodied species like daphnids and midge larvae than for organisms with a hard exoskeleton (e.g. glass shrimp and crayfish). For midge larvae, there is preliminary evidence that Bayer 73 is accumulated to some extent through the integument, for substantial residues have been found within the body wall and haemolymph after a sublethal exposure (Hansen *et al.* 1980).

Residues of ^{14}C -Bayer 2353 were cleared quickly. Sanders (1977) determined that 90% of the accumulated concentrations in the scud and midge (those species with the highest concentrations) were eliminated within 48 h of transfer to water free of ^{14}C -Bayer 2353. Kawatski and Zittel (1977) found that larvae of the midge excreted ^{14}C -Bayer 2353 during the exposure period as well as after transfer to water free of ^{14}C -Bayer 2353. The rate of elimination during the exposure period actually exceeded the uptake rate after 24 h, a fact that was attributed to the biotransforming ability of the midge. For example, during a 96-h exposure to 0.063 mg.L^{-1} ^{14}C -Bayer 2353, the accumulation peaked at $275 \text{ } \mu\text{g.g}^{-1}$ dry weight after 24 h and actually declined to $90 \text{ } \mu\text{g.g}^{-1}$ dry weight after 96 h. Two metabolites were found. One was identified as ^{14}C -chlorosalicyclic acid while the other was thought to be the conjugated form of either Bayer 2353 or chlorosalicyclic acid. The half-life of Bayer 2353 ranged from 3.5 to 25.4 h and was inversely related to the total amount accumulated; the greater the accumulation, the faster the elimination.

No data are available concerning the cycling of Bayer 73 in molluscs or oligochaetes. Both groups are very susceptible to Bayer 73 (see Section 10.3).

The bioconcentration factors calculated by Sanders (1977) (Table 5-5) may be somewhat misleading, as they are based on the wet weight of the organisms tested. However, the rapid clearance of residues that had reached equilibrium in these invertebrates, suggests that biomagnification of Bayer 73 would be unlikely to occur in the natural environment.

Fish

The uptake and distribution of Bayer 73 into different tissues in fish are given in Table 5-6. In general, the pattern of uptake is similar to that observed for TFM (Table 5-2). The uptake is rapid; 50% of the initial quantity present in the treatment tank in a static test was concentrated by rainbow trout within 24 h (Statham and Lech 1975). Dawson *et al.* (1982) found that levels in the muscle tissue and plasma peaked after 12 h of exposure in rainbow trout, channel catfish and largemouth bass (Table 5-6). The residues of Bayer 73 in muscle, heart

Table 5-5. Accumulation of ^{14}C -Bayer 2353 from water by 7 aquatic invertebrates after a 24-h exposure (water hardness = $270 \text{ mg.L}^{-1} \text{ CaCO}_3$, pH = 7.4, temperature = 22°C for crayfish and midge, 18°C for all others) (from Sanders 1977).

Species	Concentration in Water (mg.L^{-1})	Concentration in Whole Body ($\mu\text{g.g}^{-1}$ wet weight)	BCF
Daphnia, 1st instar (<u>Daphnia magna</u>)	0.0014	0.075	53
Aquatic sowbug, mature (<u>Asellus brevicaudus</u>)	0.0011	0.025	23
Scud, mature (<u>Gammarus pseudolimnaeus</u>)	0.0012	0.080	67
Glass shrimp, mature (<u>Palaemonetes kadiakensis</u>)	0.0010	0.004	4
Crayfish, juvenile (<u>Orconectes nais</u>)	0.0010	0.004	4
Damselfly, mature nymph (<u>Ischnura verticalis</u>)	0.0012	0.008	7
Midge, 4th instar (<u>Chironomus plumosus</u>)	0.0011	0.087	80

Table 5-6. Uptake and distribution of Bayer 73 in bile and different tissues of fish.

Species	Bile/ Tissue	Exposure Time (h)	Concentration of Bayer 73 in Water ($\mu\text{g}\cdot\text{L}^{-1}$)	Concentration of Bayer 73 in Tissue ($\mu\text{g}\cdot\text{g}^{-1}$)	Reference
Rainbow trout (<i>Salmo gairdneri</i>)	blood	12	0.05	4.35	Statham and Leon (1975) ^a
	heart	12	0.05	1.57	"
	muscle	12	0.05	0.08	"
	liver	12	0.05	12.5	"
	bile	12	0.05	277	"
Rainbow trout (<i>Salmo gairdneri</i>)	plasma	12	0.05	7.66 ^a	Dawson et al. (1982) ^c
		24	0.05	5.30 ^a	
	muscle	12	0.05	0.045	
		24	0.05	0.024	
	bile	12	0.05	380.0 ^a	
Largemouth bass (<i>Micropterus salmoides</i>)	blood	12	0.05	11.38	Schultz and Harman (1978b) ^d
		24	0.05	15.71	
	brain	12	0.05	0.77	
		24	0.05	0.92	
	muscle	12	0.05	0.66	
Largemouth bass (<i>Micropterus salmoides</i>)	kidney	12	0.05	0.67	Dawson et al. (1982) ^c
		24	0.05	7.24	
	liver	12	0.05	9.34	
		24	0.05	13.33	
	liver	12	0.05	12.41	
Channel catfish (<i>Ictalurus punctatus</i>)	liver	12	0.05	317.52	Dawson et al. (1982) ^c
		24	0.05	411.25	
	muscle	12	0.05	0.058	
		24	0.05	0.048	
	muscle	12	0.05	0.022	

^a $\mu\text{g}\cdot\text{mL}^{-1}$

^b water hardness = 134 $\text{mg}\cdot\text{L}^{-1}$ CaCO_3 , pH = 7.52, temperature = 12°C.

^c water hardness = 100 $\text{mg}\cdot\text{L}^{-1}$ CaCO_3 , pH = 8.2, temperature = 12°C.

^d water hardness = -, pH = 7.3, temperature = 13.5°C.

^e water hardness = 23.5 $\text{mg}\cdot\text{L}^{-1}$ CaCO_3 , pH = 7.3, temperature = 19°C.

and brain tissue are relatively low ($<2 \mu\text{g}\cdot\text{g}^{-1}$) (Table 5-6). Those in the kidney and liver are substantially higher and are indicative of sites of metabolism and excretion (Statham and Lech 1975; Allen *et al.* 1979). The extremely high levels in the bile of rainbow trout and largemouth bass suggest that Bayer 73 is metabolized into the conjugated form by the liver and then secreted into the gallbladder.

Rainbow trout are able to conjugate Bayer 73 with glucuronic acid and excrete it in the bile and the urine (Statham and Lech 1975; Allen *et al.* 1979), as is the case for TFM. Allen *et al.* (1979) administered an intraperitoneal injection of Bayer 73 to trout and found that 72 h after injection, about 20% of the injected dose was recovered in the bile, while 25% was excreted in the urine. Exposure of the fish to $0.05 \text{ mg}\cdot\text{L}^{-1}$ Bayer 73 confirmed the presence of Bayer 73 in the urine, most of which was in the conjugated form. Similarly, Statham and Lech (1975) established that after a 24-h static exposure to $0.05 \text{ mg}\cdot\text{L}^{-1}$ ^{14}C -Bayer 73, the bile-to-water ratio of ^{14}C -Bayer 73 increased to 10,000:1, and that essentially all of the residue was conjugated. The mechanism of conjugation with glucuronic acid is similar to that outlined for TFM.

The elimination of Bayer 73 by various fish tissues is illustrated in Table 5-7. After the fish were transferred to Bayer 73-free water, the concentration of Bayer 73 declined with time in the tissues and body fluids examined, except for the bile. Schultz and Harman (1978b) suggested that some of the tissue residues were transported to the gallbladder for excretion. Levels in the heart, muscle and brain dropped to $< 1 \mu\text{g}\cdot\text{g}^{-1}$ after 24 h in Bayer 73-free water. Residues in the blood, liver and kidneys had decreased, but were still detectable after 72 h. Large quantities of Bayer 73 in the urine (Allen *et al.* 1979) and the bile (Statham and Lech 1975) indicate that this compound is subject to renal and biliary excretion.

Dawson *et al.* (1982) examined the clearance of accumulated residues of Bayer 73 from the muscle, plasma and bile of rainbow trout over a 21-day period following a 12-hour exposure to $0.05 \text{ mg}\cdot\text{L}^{-1}$ Bayer 73 (Table 5-8). Levels of Bayer 73 in the muscle and plasma began to decrease 24 h after the trout were transferred to Bayer 73-free water and were below the limits of detection ($0.01 \mu\text{g}\cdot\text{g}^{-1}$) 10 and 21 days postexposure, respectively. In contrast, levels in the bile increased during the 24-h period, attaining a maximum concentration of $1180 \mu\text{g}\cdot\text{g}^{-1}$, probably as a result of residue pools continuing to empty into the bile. The concentration declined to $0.95 \mu\text{g}\cdot\text{g}^{-1}$ after 7 days and fluctuated at this level until the experiment was terminated. A residue was still present after 21 days, suggesting that Bayer 73 may have been partly resorbed and recycled into the digestive tract (Dawson *et al.* 1982).

Table 5-7. Clearance of Bayer 73 from bile and different tissues of fish up to 72 h postexposure.

Species	Bile/ Tissue	Exposure Time (h)	Concentration of Bayer 73 in Water (mg.L^{-1})	Recovery Period (h)	Concentration of Bayer 73 in Tissue ($\mu\text{g.g}^{-1}$)	Reference
Rainbow trout (<i>Salmo gairdneri</i>)	blood	12	0.05	24	0.64	Statham and Lech ^a (1975)
				72	0.27	
	heart	12	0.05	24	0.40	"
				72	0.30	
	muscle	12	0.05	24	0.02	"
				72	0.07	
Largemouth bass (<i>Micropterus salmoides</i>)	liver	12	0.05	24	3.62	"
				72	2.09	
	bile	12	0.05	24	265	"
				72	398	
	blood	24	0.05	24	4.75	Schultz and Harman ^b (1978b)
		24	0.05	72	3.56	
	brain	24	0.05	24	0.62	"
		24	0.05	72	0.44	
	muscle	24	0.05	24	0.31	"
		24	0.05	72	0.25	
	kidney	24	0.05	24	3.59	"
		24	0.05	72	3.31	
	liver	24	0.05	24	1.97	"
		24	0.05	72	1.23	
	bile	24	0.05	24	126.67	"
		24	0.05	72	293.31	

^a water hardness = $134 \text{ mg.L}^{-1} \text{ CaCO}_3$, pH = 7.52, temperature = 12°C .^b water hardness = -, pH = 7.3, temperature = 13.5°C .

Table 5-8. Clearance of Bayer 73 from bile and different tissues of fish up to 21 days postexposure (water hardness = 100 mg.L⁻¹ CaCO₃, pH = 8.2, temperature = 12°C) (from Dawson et al. 1982).

Species	Bile/ Tissue	Exposure time (h)	Concentration of Bayer 73 in Water (mg.L ⁻¹)	Recovery period (days)	Concentration of Bayer 73 in Tissue (µg.g ⁻¹)
Rainbow trout (<u>Salmo gairdneri</u>)	plasma	12	0.05	1	6.93
				3	3.35
				7	0.10
				10	0.064
				14	0.04
				21	<0.01
	muscle	12	0.05	1	0.16
				3	0.022
				7	0.010
				10	<0.01
				14	<0.01
				21	<0.01
	bile	12	0.05	1	1180
				3	117
				7	0.95
				10	1.45
				14	1.11
				21	0.76

Sea Lampreys, Amphibians, Reptiles, Birds and Mammals

No information is available concerning the uptake, distribution or elimination of Bayer 73 in sea lampreys, amphibians, reptiles, aquatic birds or aquatic mammals. The metabolism of Bayer 2353 by rats has been investigated by Griffiths and Facchini (1979). A single oral dose of 20 mg of ^{14}C -Bayer 2353 was given to adult Wistar rats (200-300 g) and the bile, urine and feces were examined for evidence of excretion. As a percentage of the dosage administered, 51.5% of the Bayer 2353 was detected in the urine, 47.4% in the feces and 1.1% in the bile. The metabolites in the urine were composed of 5.0% ^{14}C -Bayer 2353, 30.8% of 2',5-dichloro-4'-aminosalicylanilide, 13.5% of conjugated 2',5-dichloro-4'-aminosalicylanilide and 1.2% of unknowns. The metabolites in the feces were made up of 34.6% ^{14}C -Bayer 2353, 10.4% of 2',5-dichloro-4'-aminosalicylanilide and 1.8% of unknowns. A minute amount in the bile was unaltered ^{14}C -Bayer 2353.

6.0

MODE OF ACTION OF TFM AND BAYER 73

6.1 MODE OF ACTION OF TFM

The mode of action of TFM in sea lampreys and nontarget organisms remains largely undefined. In early studies, the hypotheses put forward to explain the mode of action were derived from observations on moribund lampreys. Applegate *et al.* (1961) described the condition of exposed sea lampreys as follows: "This chemical causes an excoriation of the respiratory epithelium accompanied by rupture of this tissue and enlargement of the blood vessels supplying it. Severe hemorrhaging from the respiratory capillaries often fills the gill pouches with blood. The compound is also hypotensive in its effect upon the larvae. Blood vessels become dilated, particularly the large posterior cardinal veins which lie on the dorsal wall of the coelom. These vessels, as well as other elements of the venous system, become greatly engorged with blood. These observations suggest that the lampreys suffer a general circulatory collapse aggravated by suffocation due to the presence of excessive mucous and blood in the gill pouches. Lamprey larvae appear first to be irritated, then to be narcotized, by the toxicant. Respiration becomes irregular and responses to stimuli weaken."

Christie and Battle (1963) observed similar effects of TFM on larvae of the American brook lamprey, *Lampetra lamottei*^a, including the following: distension of the pharyngeal area and emergence of heavy cords of mucus from the gill clefts, widespread vasodilation of vessels in the gill filaments, edema in the connective tissue between the respiratory epithelium and the vascular epithelium, increased production and/or discharge of mucus by mucous cells, reddish color in the cloacal region and liver due to dilation of the venous sinuses, extensive edema of the fibrous connective tissue of the skeletal musculature and a slightly increased secretory activity of mucus-secreting cells in the epidermis. These observations support the mode of action proposed by Applegate *et al.* (1961), that death occurs because of circulatory and respiratory collapse.

Recently, Mallatt and Ridgway (1984) conducted a study on ultrastructural effects of TFM on gills of larval sea lampreys. They exposed ammocoetes to a 9-h LC₅₀ concentration of 2.25 mg.L⁻¹ TFM and then examined the gills of survivors using electron microscopy. The results indicated that ion-uptake cells were universally, and almost exclusively, adversely affected. The observed changes ranged from cells that lacked only surface projections to those that contained vacuoles and showed abnormal mitochondria. Interestingly, many of the

^a Current scientific name (AFS 1980). In the original paper the scientific name was *Entosphenus lamottei*.

observations noted by Christie and Battle (1963) were absent in this study. For example, there was no evidence of vasodilation of gill blood vessels, lamellae were not congested with blood, hemorrhaging was absent and excessive mucus secretion was not apparent. However, since the concentration of TFM used in this study was not lethal to the organisms examined, the results may tend to reflect the mode of cellular toxicity, but not necessarily the cause of death. On the other hand, some of the effects reported by Christie and Battle (1963) may have been postmortem changes.

Rainbow trout were also exposed to lethal concentrations of TFM under identical conditions as the lampreys in the respective studies of Applegate *et al.* (1961) and Christie and Battle (1963). In contrast to the mode of action hypothesized for the lampreys, the fish exhibited excessive mucus production by the gills and classical signs of anoxia. Sea lamprey do not exhibit signs of anoxia when moribund (Agris 1967).

At the biochemical level, it was proposed that on the basis of structural similarities with 2,4 dinitrophenol (DNP: the classical uncoupler of oxidative phosphorylation) TFM could act in the same manner (Applegate *et al.* 1966). Indeed, there is some evidence to support this concept based on observations of stimulation of oxygen consumption in bacteria (Liu *et al.* 1977), aquatic invertebrates (Kawatski *et al.* 1974), sea lampreys (Smith and King 1969) and rat liver mitochondria (Niblett and Ballantyne 1976). For example, Liu *et al.* (1977) tested similar concentrations of DNP and TFM and compared their ability to stimulate oxygen consumption in the sediment bacterium *Acinetobacter* sp. On this basis, TFM was found to be as potent an uncoupler of oxidative phosphorylation as DNP. Niblett and Ballantyne (1976) determined in an *in vitro* study of rat liver mitochondria, that TFM was a more potent uncoupler than DNP. Similarly, Kawatski *et al.* (1974) reported that TFM at concentrations of 2 to 8 mg.L⁻¹ increased the oxygen uptake of larval chironomids *in vivo* during a 6-h experiment. However, as well as being a possible uncoupler, it was also suggested that TFM was eliciting an increase in oxygen consumption in response to a demand for energy needed for the biotransformation activity and excretion of TFM conjugates (Kawatski *et al.* 1974).

The results in fish were different with respect to oxygen consumption. Smith and King (1969) found that the oxygen uptake was not increased in rainbow trout exposed to TFM. Kawatski and McDonald (1974) determined that concentrations of 1 mg.L⁻¹ TFM or greater significantly reduced the oxygen consumption of liver homogenates of white sucker, largemouth bass, bluegill sunfish and rainbow trout. The stimulation of oxygen utilization typically seen at certain low concentrations of uncouplers was seen only in trout brain and bluegill liver, and the stimulation in bluegill liver occurred only when the homogenate protein concentration was high. Thus, with the exception of trout brain, TFM does not appear to be acting as an uncoupler of oxidative phosphorylation in fish.

Hunn (1971) showed that adult white suckers exposed to 5 mg.L⁻¹ TFM exhibited such an increase in plasma concentrations of glucose and lactic acid that after a 6-h exposure, most fish were moribund because of lactic acidosis. This increase in plasma concentrations of glucose and lactic acid in white suckers together with the observation that TFM decreases liver oxygen utilization in vitro (Kawatski and McDonald 1974) indicates that TFM inhibits enzymes of the terminal respiratory pathways, causing blockage at or near the level of pyruvate.

6.2 MODE OF ACTION OF BAYER 73

The mode of action of Bayer 73 has also not been elucidated, although it appears to act in a fashion similar to TFM. Smith and King (1969) found that sublethal levels of Bayer 73 stimulated oxygen consumption in the sea lamprey, but not in rainbow trout. The oxygen uptake of the aquatic midge, Chironomus tentans, was dependent on the concentrations of Bayer 73 to which it was exposed (Kawatski et al. 1974). Concentrations between 0.75 and 1.00 mg.L⁻¹ stimulated oxygen consumption significantly whereas concentrations of 1.20 mg.L⁻¹ or greater inhibited oxygen consumption, suggesting that Bayer 73 was acting as an uncoupler of oxidative phosphorylation. Ishak et al. (1970) observed similar results in their study of snail tissue homogenates subjected to Bayer 73 and sodium pentachlorophenate, and concluded that at low concentrations these compounds uncoupled oxidative phosphorylation while at higher levels, glycolysis was inhibited.

As was observed for TFM, Kawatski et al. (1974) also suggested that the increased oxygen consumption by the midge at low levels of Bayer 73 could also represent the activation of an energy-requiring, detoxification mechanism. Thus, Bayer 73 may be acting in a similar manner as TFM, although the exact mechanism is unknown.

6.3 SELECTIVITY OF TFM

It has been proposed that the selectivity of TFM, with regard to teleost fish, is due to the biotransformation ability of teleosts, which is apparently lacking in lampreys (see Section 5.2.2.1). Through the use of inhibitors of glucuronide formation, it has been demonstrated that this mechanism is important in the protection of fish from the toxic effects of TFM (Lech et al. 1973; Lech 1974). For example, when rainbow trout were injected with novobiocin, a known inhibitor of glucuronidation, at a dosage of 200 mg.kg⁻¹, the 3-h LC₅₀ declined from 5.20 mg.L⁻¹ to 2.48 mg.L⁻¹ (Lech et al. 1973). Similarly, Lech (1974) found that exposure to another inhibitor, salicylamide, at a concentration of 25 mg.L⁻¹, resulted in a decline in the 4-h LC₅₀ from 5.05 to 2.67 mg.L⁻¹ for rainbow trout. Coincident with the observed increase in toxicity were elevated levels of unconjugated TFM in the blood, brain, muscle and heart and reduced levels of TFM glucuronide in

the blood and bile. In addition, the glucuronidation of TFM by liver extracts in vitro of salicylamide-exposed trout was inhibited.

Lech and Statham (1975) confirmed the protective effect of TFM conjugation in a comparative study of various life stages of sea lampreys and rainbow trout. Not only were the concentrations of TFM much higher in the blood and tissues of all stages of the sea lamprey, but an in vitro study of the liver revealed a very limited capacity of lampreys to conjugate TFM (see Section 5.2.2.1). These observations were corroborated by the results of an acute toxicity test. Trout exposed to a range of concentrations of TFM had a 24-h LC_{50} of 9.7 mg.L^{-1} as opposed to only 2.0 mg.L^{-1} for the lampreys. However, after a 2-h exposure to 25 mg.L^{-1} salicylamide, the 24-h LC_{50} dropped to 3.6 mg.L^{-1} for the trout, but remained relatively unchanged at 2.2 mg.L^{-1} for the lampreys. It appears then that factors which contribute to the susceptibility of sea lampreys to TFM are related to their lower capacity to conjugate TFM and a greater net uptake of TFM, as compared with those of rainbow trout.

Data concerning the selectivity of TFM relative to aquatic invertebrates suggest that TFM is nontoxic to most invertebrate species because of their limited uptake and rapid excretion of the chemical (see Section 5.2.2.1 and Table 10-1). In addition, some species may be able to detoxify TFM by transforming it to other less toxic compounds (Kawatski and Bittner 1975).

Although Bayer 73 is more than 43 times as toxic as TFM to larval lampreys, it is not used alone to treat streams because it is essentially nonselective between lampreys and rainbow trout (Howell et al. 1964). Marking and Hogan (1967) found that Bayer 73 was also highly toxic to a number of other fish species. The reader is referred to section 3.2.1 concerning the use of Bayer 73.

7.0 THE EFFECT OF ENVIRONMENTAL FACTORS ON THE TOXICITY OF TFM, BAYER 73 AND A 98:2 MIXTURE

Data concerning the effects of environmental factors on the toxicities of TFM, Bayer 73 and the 98:2 mixture are based on 96-h LC_{50} tests. Although this exposure period does not accurately reflect those used in the field, the data can be used for comparative purposes, since they illustrate the effects of temperature, hardness and pH on lampricide toxicity.

Water hardness and alkalinity have both been used to characterize streams treated with TFM. If the hardness is due to the presence of carbonates, there is a direct relationship between the two, i.e. as hardness increases so does the alkalinity. In such instances, either characteristic can be used to predict effects on the activity of TFM. If the water hardness is due to other salts, such as chlorides, there is less correlation between hardness and alkalinity; the latter may serve as the better predictive indicator. Field treatment crews now generally use total alkalinity rather than hardness in determining the concentrations to be applied. However, laboratory studies investigating the effects of environmental factors on lampricide toxicity have concentrated on total hardness, rather than total alkalinity, and hence the data presented in this section reflect this trend.

With regard to the TFM:Bayer 73 mixture, ratios used in the field may vary from 99.5:0.5 to 98:2. Because most laboratory data have been derived using a ratio of TFM:Bayer 73 of 98:2 for comparative purposes, the following discussion is based on that combination.

7.1 EFFECT OF TEMPERATURE, HARDNESS AND pH ON THE TOXICITY OF TFM

7.1.1 EFFECT OF TEMPERATURE

Temperature does not exert a significant effect on the toxicity of TFM to sea lamprey larvae based on laboratory data. Although the 96-h LC_{50} values in Table 7-1 fluctuated somewhat between 7 and 17°C (at the same pH and hardness), Dawson *et al.* (1977) concluded that they were not significantly different. These results confirmed earlier work under identical conditions (Dawson *et al.* 1975). However in the field it has been reported at low water temperatures ($\leq 4^\circ\text{C}$) the response of burrowed ammocoetes is delayed, possibly because of a reduced metabolic rate (Smith *et al.* 1974).

In coldwater fish an increase in temperature appears to increase the toxicity of TFM slightly (Table 7-2). For example, as the temperature increased from 7 to 17°C in water of constant hardness (44 mg.L^{-1} CaCO_3) and pH (7.5), the 96-h LC_{50} for rainbow trout declined from 2.13 to 1.74 mg.L^{-1} . Marking and Olson (1975) observed the same effect in rainbow trout as well as in other coldwater species such as

Table 7-1. Effects of temperature, hardness and pH on the 96-h LC₅₀ of formulated TFM^a, Bayer 73^b and the 98:2^c mixture to the sea lamprey (from Dawson et al. 1977).

Temp. (°C)	Hardness (mg.L ⁻¹ CaCO ₃)	pH	96-h LC ₅₀			
			Individual		98:2 Mixture	
			TFM	Bayer 73	TFM	Bayer 73
			and 95% confidence interval	and 95% confidence interval	and 95% confidence interval	and 95% confidence interval
			(mg.L ⁻¹)	(mg.L ⁻¹)	(mg.L ⁻¹)	(mg.L ⁻¹)
7	44	7.5	0.345 (0.246-0.484)	0.0220 (0.0158-0.0306)	0.440 (0.345-0.561)	0.00898 (0.00704-0.0114)
12	44	7.5	0.730 (0.612-0.870)	0.0400 (0.0268-0.0598)	0.375 (0.255-0.551)	0.00765 (0.00520-0.0112)
17	44	7.5	0.496 (0.415-0.593)	0.0410 (0.0309-0.0540)	0.455 (0.350-0.591)	0.00928 (0.00715-0.0121)
12	170	7.5	0.625 (0.488-0.802)	0.0350 (0.0249-0.0491)	0.560 (0.435-0.721)	0.01140 (0.00888-0.0147)
12	300	7.5	0.710 (0.530-0.951)	0.0390 (0.0266-0.0571)	0.560 (0.449-0.699)	0.01140 (0.00920-0.0143)
12	44	6.5	0.172 (0.121-0.245)	0.0300 (0.0222-0.0406)	0.170 (0.121-0.239)	0.00350 (0.00250-0.00490)
12	44	8.5	1.30 (0.957-1.77)	0.0390 (0.0266-0.0570)	0.580 (0.453-0.743)	0.0118 (0.00920-0.0152)

^a TFM is a 35.7% by weight active ingredient formulation.

^b Bayer 73 is a 70% by weight active ingredient formulation.

^c 98:2 is a mixture of TFM and Bayer 73 in the proportion 98:2 by weight, based on active ingredient.

Table 7-2. Effects of temperature, hardness and pH on the 96-h LC₅₀ of formulated TFM^a, Bayer 73^b and the 98:2^c mixture to rainbow trout (from Bills and Marking 1976).

Temp. (°C)	Hardness (mg.L ⁻¹ CaCO ₃)	pH	96-h LC ₅₀			
			Individual		98:2 Mixture	
			TFM and 95% confidence interval	Bayer 73 and 95% confidence interval	TFM and 95% confidence interval	Bayer 73 and 95% confidence interval
			(mg.L ⁻¹)	(mg.L ⁻¹)	(mg.L ⁻¹)	(mg.L ⁻¹)
7	44	7.5	2.13 (1.73-2.62)	0.0620 (0.0568-0.0677)	1.42 (1.15-1.76)	0.0290 (0.0234-0.0358)
12	44	7.5	1.81 (1.53-2.14)	0.0346 (0.0297-0.0404)	1.16 (0.998-1.35)	0.0237 (0.0204-0.0275)
17	44	7.5	1.74 (1.33-2.27)	0.0439 (0.0396-0.0487)	1.41 (1.14-1.74)	0.0288 (0.0232-0.0366)
12	10	8.1	9.00 (8.13-9.96)	0.0800 (0.0656-0.0984)	3.89 (3.18-4.76)	0.0794 (0.0649-0.0970)
12	44	8.1	14.1 (11.4-17.4)	0.0755 (0.0649-0.0878)	3.62 (3.16-4.14)	0.0738 (0.0646-0.0845)
12	160	8.1	14.1 (11.4-17.4)	0.100 (0.0817-0.122)	4.85 (4.03-5.83)	0.0989 (0.0823-0.119)
12	300	8.1	17.3 (14.0-21.4)	0.0865 (0.0756-0.0990)	4.65 (3.90-5.54)	0.0949 (0.0796-0.113)
12	44	6.5	0.949 (0.840-1.07)	0.0261 (0.0197-0.0345)	0.750 (0.699-0.841)	0.0153 (0.0136-0.0172)
12	44	8.5	5.40 (4.58-6.37)	0.0705 (0.0552-0.0901)	3.70 (3.09-4.43)	0.0755 (0.0630-0.0898)
12	44	9.5	25.2 (20.4-31.1)	0.185 (0.133-0.257)	9.30 (8.20-10.5)	0.1900 (0.167-0.215)

^a TFM is a 35.7% by weight active ingredient formulation.

^b Bayer 73 is a 70% by weight active ingredient formulation.

^c 98:2 is a mixture of TFM and Bayer 73 in the proportion 98:2 by weight based on active ingredient.

chinook salmon, brown trout (Salmo trutta) and lake trout. However, for warmwater species, with the exception of the bluegill, an increase in temperature from 12 to 22°C had no effect on the toxicity of TFM to channel catfish (Ictalurus punctatus), yellow perch (Perca flavescens) and carp (Cyprinus carpio). Hence, it appears that an increase in temperature can be expected to increase slightly the toxicity of TFM to coldwater species, but would have no effect on most warmwater species.

If fish are already stressed by excessively high or fluctuating temperatures, any added stress, such as a lampricide treatment, can lead to fish mortality. For example, in the Nottawasaga River, TFM applied at a "normally safe" concentration produced a heavy mortality in suckers spp., carp and walleyes (Lamsa *et al.* 1969). In addition, pike, bowfins (Amia calva), alewives, burbot and species of bass were killed. In the Muskegon River, a large number of white suckers and walleyes died when the water temperature suddenly increased about 5 C° during the course of a treatment (Smith and King 1969). It appears that temperatures within "normal ranges" have little effect on the toxicity of TFM. But, if temperature is acting as a stressing factor, it tends to make fish more susceptible to TFM, and probably to other toxicants as well.

In his study of the effect of temperature on the toxicity of TFM to invertebrates, Fremling (1975) established that temperature had no effect on the toxicity of TFM to mayfly nymphs.

7.1.2 EFFECT OF WATER HARDNESS

The effect of hardness on the toxicity of TFM to lampreys is related to pH. At 12°C and pH 7.5, Dawson *et al.* (1977) found that as the hardness increased from 44 to 300 mg.L⁻¹, the 96-h LC₅₀ remained virtually unchanged (Table 7-1). Dawson *et al.* (1975) observed the same effect at pH 6.5. However at pH 8.5, an increase in hardness from 12 to 300 mg.L⁻¹ CaCO₃ decreased the toxicity of TFM, i.e. the 96-h LC₅₀ increased from 1.17 to 3.57 mg.L⁻¹.

Bills and Marking (1976) established that increasing the water hardness decreased the toxicity of TFM to fish. For instance, as the hardness increased from 10 to 300 mg.L⁻¹ CaCO₃ at a constant pH of 8.1, the 96-h LC₅₀ of rainbow trout increased from 9.00 to 17.3 mg.L⁻¹ (Table 7-2). A similar effect was also noted for several species of fish including coho salmon, brown trout, lake trout, carp, channel catfish, bluegill and yellow perch (Marking and Olson 1975), as well as for early life stages of salmonids (Olson and Marking 1973, 1975).

Hardness has a protective effect for invertebrates (Kawatski *et al.* 1974, 1975), which is apparently related to a reduced accumulation of TFM at elevated levels of hardness. Kawatski and Bittner (1975) found that after a 24-h exposure to 0.075 mg.L⁻¹ TFM in

laboratory beakers, the fourth instar larvae of the aquatic midge, Chironomus tentans, had residue levels of 30 and 125 $\mu\text{g.g}^{-1}$ dry weight at hardnesses of 280-320 and 40-48 mg.L^{-1} CaCO_3 , respectively. Exposure to TFM alone or in a 98:2 mixture had no effect on the uptake levels. The elimination of TFM was also related to water hardness; the harder the water, the faster the rate of elimination.

7.1.3 EFFECT OF pH

The toxicity of TFM is affected to the greatest extent by pH; as the pH increases, the toxicity decreases. This effect was initially reported for sea lamprey larvae and rainbow trout in an early study by Lemaire (1961). It has since been confirmed (Bills and Marking 1976; Dawson *et al.* 1977). For example, the 96-h LC_{50} for sea lampreys increased from 0.172 mg.L^{-1} at pH 6.5 to 1.30 mg.L^{-1} at pH 8.5, a factor of nearly 8 times (Table 7-1). Similarly, for rainbow trout the 96-h LC_{50} was more than 25 times greater at pH 9.5 than at 6.5 (Table 7-2). Marking and Olson (1975) observed the same effect in coho salmon, brown trout, lake trout, carp, channel catfish, bluegill and yellow perch. The increased toxicity at low pH values is presumably caused by a greater uptake. Sills and Allen (1975), examined the accumulation of TFM in the muscle of exposed channel catfish and found that at a constant hardness of 45 mg.L^{-1} CaCO_3 and temperature of 18.5°C, the accumulation of TFM increased from 0.03 $\mu\text{g.g}^{-1}$ at pH 9 to 3.21 $\mu\text{g.g}^{-1}$ at pH 6, an increase of more than 100 times.

Fremling (1975) observed that mayfly nymphs were similarly affected. Based on 96-h LC_{50} data, TFM was 50 times more toxic at pH 6.5 than at pH 9.5. For example, at a hardness of 40-48 mg.L^{-1} CaCO_3 , and a temperature of 22-23°C, the 96-h LC_{50} increased from 0.52 mg.L^{-1} TFM at pH 6.5 to 26.3 mg.L^{-1} TFM at pH 9.5.

7.2 EFFECT OF TEMPERATURE, HARDNESS AND pH ON THE TOXICITY OF BAYER 73

7.2.1 EFFECT OF TEMPERATURE

Based on laboratory data, the toxicity of Bayer 73 to lampreys, although slightly greater at 7 than at 17°C, was not significantly altered by temperature (Table 7-1). However, during stream treatments at low temperatures ($\leq 4.4^\circ\text{C}$) the efficacy of Bayer 73 is reduced (Smith *et al.* 1974). In teleost fish, the toxicity of Bayer 73 was affected very little by temperature (Marking and Hogan 1967; Bills and Marking 1976). The 96-h LC_{50} did not change markedly between 7 and 17°C for rainbow trout (Table 7-2) or between 12 and 22°C for goldfish and channel catfish (Marking and Hogan 1967). However Bayer 73 was moderately more toxic to bluegills at 22°C (96-h LC_{50} = 0.054 mg.L^{-1}) than at 12°C (96-h LC_{50} = 0.092 mg.L^{-1}). No information is

currently available regarding the effect of temperature on the toxicity of Bayer 73 to invertebrates.

7.2.2 EFFECT OF WATER HARDNESS

As illustrated in Tables 7-1 and 7-2, hardness had no effect on the 96-h LC_{50} values of Bayer 73 to lampreys and rainbow trout, respectively. Similarly, Olson and Marking (1975) noted that hardness did not affect the toxicity of Bayer 73 to green eggs of coho salmon. In contrast, Marking and Hogan (1967) observed that with increasing hardness there was a slight decrease in the toxicity of Bayer 73 to rainbow trout and bluegill sunfish. For example, at hardness levels of 44 and 170 $mg.L^{-1}$ $CaCO_3$, the 96-h LC_{50} increased from 0.053 to 0.070 $mg.L^{-1}$ for trout and from 0.068 to 0.117 $mg.L^{-1}$ for bluegills. However, the pH of the test water also increased from 7.4 to 7.8 as the hardness rose. Hence, it is quite possible that the observed decrease in toxicity was due to the effect of pH.

With regard to invertebrates, it appears that the toxicity of Bayer 73 is also independent of hardness. For example, although Kawatski and Zittel (1977) found that the uptake of Bayer 73 by the aquatic midge was initially enhanced in soft water, this apparent effect disappeared after longer exposures of 24 to 96 h. Kawatski et al. (1975) determined that the toxicity of Bayer 73 to the aquatic midge decreased about 1.5 times as the hardness rose from 40-48 to 280-320 $mg.L^{-1}$. But, the pH also increased from 7.2-7.6 to 8.0-8.4 with the change in hardness, and consequently they were unable to discern whether hardness or pH was responsible for the observed difference.

7.2.3 EFFECT OF pH

Although pH had the most significant effect on the toxicity of Bayer 73, its influence was less pronounced than on TFM. Dawson et al. (1977) determined that with an increase in pH from 6.5 to 8.5, the 96-h LC_{50} increased 1.3 times for the sea lamprey (Table 7-1). For rainbow trout (Table 7-2) Bayer 73 was 7 times more toxic at pH 6.5 than at pH 9.5. The only available study concerning invertebrates and the effect of pH on Bayer 73 toxicity is that of Kawatski et al. (1975) (see Section 7.2.2).

It should be pointed out that although Bayer 73 is generally more toxic at lower pHs, it becomes insoluble once the pH reaches a certain level. Meredith (1971a), for example, reported that the toxicity of Bayer 73 as a molluscicide declined at pH values below 7, because of precipitation. Similarly, Bayer 73 was found to precipitate below pH 6.5 (NFRL 1983a).

7.3 EFFECT OF TEMPERATURE, WATER HARDNESS, pH AND DISSOLVED OXYGEN ON THE TOXICITY OF A 98:2 MIXTURE OF TFM AND BAYER 73

The toxicity of the 98:2 mixture to sea lampreys and rainbow trout as defined by the additive index principle of Marking and Dawson (1975) (see Section 11.4) is additive or less than additive (Bills and Marking 1976; Dawson et al. 1977). From Table 7-2 it can be seen that the amount of Bayer 73 used alone and in the mixture to effect the same degree of kill is approximately the same, whereas for TFM, the amount required in the mixture is generally 2-3 times less. Thus, although there is no toxicological advantage, it is economically beneficial to use the mixture (see Section 3.2.1).

Although temperature and hardness have little or no effect on the toxicity of the mixture to ammocoetes and teleosts (Tables 7-1 and 7-2), pH can exert a considerable influence. For example, the mixture was more than three times as toxic to sea lampreys at pH 6.5 than at pH 8.5 (Table 7-1). Similarly, it was more than 12 times as toxic to rainbow trout at pH 6.5 than at pH 9.5 (Table 7-2). Kawatski et al. (1975) found that decreasing hardness accompanied by a lower pH increased the toxicity of the mixture to the aquatic midge. No data are available concerning the effect of temperature on the toxicity of the mixture to invertebrates.

Low dissolved oxygen levels ($<4 \text{ mg.L}^{-1}$) may stress fish (Doudoroff and Shumway 1970; NRCC 1975) and make them more susceptible to the action of the 98:2 mixture. For instance, the treatment of the Kalamazoo River, a tributary of Lake Michigan which had dissolved oxygen concentrations ranging from 0.6 to 2.4 mg.L^{-1} , with a theoretically "safe concentration" of the 98:2 mixture resulted in the loss of large numbers of carp, suckers spp., channel catfish, northern pike, alewife, gizzard shad (Dorosoma cepedianum) and longnose gar (Lepisosteus osseus) (Smith 1967b). Presumably, low dissolved oxygen concentrations would increase the susceptibility to other lampricides as well.

7.4 MECHANISM OF ACTION OF pH AND WATER HARDNESS

The hardness and pH of the exposure water are thought to affect the toxicity of TFM by determining the form in which the molecule exists (Howell et al. 1980). The pK_a of TFM was reported to be 6.07 (Applegate et al. 1961). Hunn and Allen (1974) have indicated that at pHs above 6.07, concentrations of the lipid-soluble free phenol form of TFM will decrease while those of the phenolate ion will increase. Because the amount of available free phenol is reduced at higher pHs, there is less uptake and consequently, less toxicity to both larval lampreys and nontarget organisms.

As the hardness increases, the increased concentration of divalent ions (Ca^{2+}) would tend to complex the ionized form of TFM. Since the phenolate ion and complex ions apparently do not cross biological gill membranes, the toxicity would decrease (Howell et al. 1980).

8.0

EFFECTS OF LAMPRICIDES ON LAMPREYS

8.1 ACUTE TOXICITY OF TFM TO NATIVE LAMPREYS AND SEA LAMPREYS

Four indigenous lamprey species inhabit tributaries of the Great Lakes: the American brook lamprey (Lampetra lamottei), the northern brook lamprey (Ichthyomyzon fossor), the silver lamprey (Ichthyomyzon unicuspis) and the chestnut lamprey (Ichthyomyzon castaneus). The chestnut lamprey is confined largely to Lake Michigan tributaries (Hubbs and Lagler 1957). The American and northern brook lampreys are nonparasitic in contrast to the silver and chestnut species. However, the latter two species are smaller and fewer in number than the sea lamprey and apparently cause no serious damage to fish populations (Scott and Crossman 1973).

Native lampreys, such as the American brook lamprey and Ichthyomyzon spp. (data on Ichthyomyzon species are lumped together because as larvae, they are difficult to distinguish), have been shown to be more resistant to TFM than sea lampreys (Davis 1970; Dawson et al. 1975). More recent studies, using different temperature and alkalinity regimes, have confirmed the greater susceptibility of the sea lamprey. For instance, the 9-h LC_{50} values for the genera Petromyzon, Ichthyomyzon and Lampetra (at 7°C and a total alkalinity of 45 $mg.L^{-1}$ $CaCO_3$) were 1.4, 2.0 and 2.5 $mg.L^{-1}$ TFM respectively (HBBS 1983). No data involving the toxicity of Bayer 73 or the TFM:Bayer 73 mixture to different genera of lampreys are available.

8.2 EFFECTS ON NATIVE LAMPREYS

8.2.1 EFFECTS ON POPULATIONS OF NATIVE LAMPREYS

Dawson et al. (1975) pointed out that although the American brook lamprey was more resistant than the sea lamprey to TFM, the difference was insufficient to permit the selective removal of only the sea lamprey. Since the chemical treatment program was initiated, there has been a decline in populations of native lampreys. Ichthyomyzon spp. have disappeared from 41 of 81 treated streams of Lake Superior (Schuldt and Goold 1980). The parasitic silver lampreys have been particularly susceptible since they tend to inhabit the lower reaches of streams which are subject to lampricide treatments.

The American brook lamprey population has not been reduced as drastically. Its disappearance was noted from only 6 of 42 treated streams in Lake Superior (Schuldt and Goold 1980). In addition, some streams that are treated only occasionally have been repopulated. In frequently treated streams, however, the total number of larvae was reduced markedly and the distribution greatly decreased. For example, between 1959 and 1975, the Two Hearted River was treated six times and the total number of larvae collected dropped by more than 80%, while the abundance declined in eight out of nine sites sampled (Schuldt and Goold

1980). It has been proposed that the American brook lampreys have not been as adversely affected as Ichthyomyzon spp. by lampricide treatments because they often inhabit headwater areas not invaded by sea lampreys, and hence are not subject to treatment (Schuldt and Goold 1980).

8.3 EFFECTS ON SEA LAMPREYS

8.3.1 EFFECTS ON THE POPULATION OF SEA LAMPREYS

Historically, estimates of the relative abundance of sea lampreys have been based on collections of spawning-phase adults taken in mechanical traps, weirs or electrical barriers installed in streams. In addition, sea lamprey abundance has been inferred from changes in counts of newly-metamorphosed downstream migrants, numbers of ammocoetes collected and the incidence of wounded and scarred fish.

The Great Lakes Fishery Commission decided to place emphasis on Lake Superior with respect to control of sea lampreys since lake trout populations had virtually disappeared from Lakes Michigan and Huron, whereas some stocks were still present in Lake Superior (Smith 1971). Consequently, most of the available information on changes in fish populations, as a result of lamprey control, is from this lake.

Counts of upstream migrating adult sea lampreys were made at electrical barriers on the same eight streams tributary to Lake Superior, from 1957 to 1978 inclusive (Smith and Tibbles 1980). After the first four years of treatment, the number of spawning-phase adults in 1962 declined approximately 80% from the previous four-year average. Thereafter, as a result of periodic scheduled applications of lampricides to prevent escapement of sea lampreys from repopulated streams, the numbers declined to about 10% of their peak abundance in 1961. Coincident with the initial drop in numbers, Smith (1971) reported that the incidence of scarring on large rainbow trout (> 30.5 cm) dropped from 13.6% in 1960 to 3.1% in 1962. He also noted reduced numbers of newly-metamorphosed migrants and ammocoetes.

No assessment barriers were operated on Lake Michigan tributaries. However, Hodges (1972) examined the numbers of recently transformed sea lampreys from the Pere Marquette River (a tributary of Lake Michigan) taken in a collection basket at a water intake structure from 1962 to 1968, and found that the average annual catch of downstream migrants dropped 99.5% after the stream was treated with TFM in 1964. Similarly, Torblaa and Westman (1980) reported that recently metamorphosed individuals caught in fyke nets set in several tributaries on the north and west shores of Lake Michigan had disappeared altogether by 1978. They also noted that the population of young parasitic sea lampreys in the Ocqueoc River, a Lake Huron tributary, had dropped 91.5% by 1973.

In Lake Ontario, weirs and traps were not installed in tributaries before chemical control was initiated in 1972, making it difficult to document the degree of effectiveness of treatment. The only available source information came from the annual counts of spawning-run lampreys captured by dip nets at night in the Humber River, at the base of a dam in Toronto between 1968 and 1978. The numbers collected by this inefficient method did not indicate a reduction in the numbers of lampreys.

Data on the percentage of salmonids showing sea lamprey attack marks have also been used to indicate the size of the sea lamprey population. The decline in wounding rates from 1970 to 1978 in Lakes Michigan and Huron is suggestive of a reduction in numbers of sea lampreys (Smith and Tibbles 1980). On the other hand, the number of planted teleosts had increased substantially during the same period, so that the apparent decline in the sea lamprey population (as indicated by a lower scarring rate) may have only reflected an increased biomass of fish, relative to a stable sea lamprey population. In Lake Ontario, the percentage of attack marks on chinook and coho salmon also declined markedly between 1971 and 1978 (Pearce *et al.* 1980). In addition, the average size of both species had increased dramatically and large numbers of age III and older chinook salmon had begun to appear, suggesting an increased survival of older and larger fish. Since sea lampreys prefer large coldwater hosts, this information does provide some evidence of a decline in sea lamprey numbers.

Data on the wounding and scarring of salmonid stocks up to 1979 indicated that lamprey predation in Lake Erie was not serious (Pearce *et al.* 1980). This was probably because of the lack of good spawning habitat in tributary streams and a limited availability of prey species. However, as pollution abatement programs improve stream habitat and as salmonid stocking and management programs become more successful, the sea lamprey could become a serious problem in Lake Erie (Pearce *et al.* 1980).

In slow-moving waters, granular Bayer 73 has been used successfully in reducing numbers of sea lamprey ammocoetes. For example, the delta of the Big Garlic River in Saux Head Lake supported a large ammocoete population, which could not be reached by TFM treatments. A 5% granular formulation, applied to an area of 5 574 m² at a rate of 123.6 kg.ha⁻¹, resulted in an 89% reduction of ammocoetes (Manion 1969).

8.3.2 EFFECTS ON DEVELOPMENTAL STAGES

Piavis (1961) determined that as the sea lamprey develops from a zygote to an ammocoete, it passes through 18 stages in a maximum period of 40 days. It was established that eggs and embryos were more resistant than ammocoete larvae to TFM and that resistance declined as

development progressed. For instance, Dawson et al. (1975) determined that the 24-h LC_{50} decreased from 3.57 mg.L^{-1} at stage 14 (hatching) to 0.56 mg.L^{-1} at stage 18 (7-cm larvae). However, exposure to TFM during embryonic development greatly increased the frequency of abnormalities at later stages and often resulted in delayed mortality. Piavis and Howell (1975) exposed stages 1 to 17 of developing lampreys to 10 mg.L^{-1} TFM for 24 h and found that with the exception of a few normal larvae that developed at stages 3, 5 and 7, all embryos became grossly abnormal and/or died (Table 8-1). Many embryos exposed during stages 1 through 7 continued to develop, but exhibited delayed hatching. Upon hatching, the embryos were grossly deformed in the shape of the letters C, J, P or O and could not straighten out, burrow or swim normally.

During normal development, hemoglobin synthesis is initiated in stage 15. In exposed embryos, hemoglobin was not detected at this stage, and it was thought that this deficiency inhibited further development. In individuals that managed to attain stage 16, the normal velum movements and heart beat decreased and the gallbladder became greatly distended.

Overall, the production of viable stage 17 prolarvae was severely reduced from a value of 71% in controls to only 4% and 0% in individuals exposed to TFM during stages 1 to 7 and 8 to 17, respectively.

8.3.3 SENSITIVITY OF BURROWED AND FREE-SWIMMING LARVAE

Contrary to the results of a preliminary report by Applegate et al. (1958), who found no difference in the sensitivity of larval lampreys in test jars and in simulated stream treatments, it has been established that burrowed lampreys are more resistant to lampricides than free-swimming larvae (Dawson et al. 1975, 1977). For TFM, the 12-h LC_{50} nearly doubled from 3.00 mg.L^{-1} for free-swimming larvae to 5.39 mg.L^{-1} for burrowed ones. Similarly, with respect to the 12-h LC_{50} for Bayer 73, free-swimming lampreys (0.092 mg.L^{-1}) were about three times more susceptible than burrowed individuals (0.280 mg.L^{-1}). The difference was even more pronounced when the 98:2 mixture was applied. Free swimming larvae were more than seven times as susceptible as burrowed lampreys (Dawson et al. 1977). Although the reasons for this difference have not been definitely established, it is thought that the free-swimming larvae are more active than burrowed ones, and hence take up more of the lampricide (Dawson et al. 1977). In addition, burrowed individuals are somewhat protected in the substrate.

8.3.4 EFFECTS ON SIZE

Since the introduction of lampricide treatments in the Great Lakes when sea lampreys were at peak abundance, the average size of

Table 8-1. Effect of TFM on developing sea lampreys: percentage of normal stage - 18 larvae produced after lampreys had been exposed at different developmental stages to 10 mg.L⁻¹ TFM for 24 h at 18.4°C (from Plavis and Howell 1975).

Stage at exposure	Number exposed	Most advanced stage attained	Percentage of surviving stage-18 larvae
1	354	17	0
2	275	17	0
3	369	18	19.0
4	199	17	0
5	286	18	1.4
6	603	17	0
7	611	18	5.7
8	275	17	0
9	246	16	0
10	252	17	0
11	212	16	0
12	314	16	0
13	200	17	0
14	203	15	0
15	245	15	0
16	160	16	0
17	50	17	0
TOTAL	4,854	-	2.2
Control 1 ^a	3,939	18	68.1
Control 2 ^a	2,509	18	74.9

^a Lampreys exposed at developmental stages 1 to 7 and 17 were from the same parents as those from Control 1; the animals exposed at stages 8-16 were from the same parents as those from Control 2.

ammocoetes (Purvis 1979), recently transformed individuals (Hodges 1972) and adults (Smith et al. 1974; Heinrich et al. 1980) has increased. This effect is probably due to three interrelated factors: lamprey abundance, availability of host species and the effectiveness of the control program (Heinrich et al. 1980). In 1954, before the advent of lampricide treatments, when food was still abundant for feeding lampreys in Lake Superior, the average length and weight were 456 mm and 218 g, respectively. As the abundance of lampreys increased and the number of hosts declined, the average size of the adults declined to 408 mm and 133 g in 1961. After the control program began, the numbers of lampreys dropped dramatically and, together with salmonid restocking efforts, the average size increased again so that in 1978 the mean length and weight were 427 mm and 161 g, respectively (Heinrich et al. 1980). Similarly, Hodges (1972) observed that recently metamorphosed individuals in a tributary of Lake Michigan increased from 134 mm in length before treatments in 1964, to 163 mm after treatments in 1968. Purvis (1979) found that after a lampricide treatment the next established class of ammocoetes grew faster, probably because of reduced density and competition.

8.3.5 EFFECTS ON SEX COMPOSITION

Before lampricide treatments began, the sex ratio of sea lamprey larvae in the upper Great Lakes was highly variable and was apparently related to changes in the size of the lamprey population (Heinrich et al. 1980). After the advent of lampricide treatments, a significant reduction in the adult population occurred, accompanied by a decline in the percentage of males (Torblaa and Westman 1980). Smith (1971) found a positive correlation between the relative abundance and the percentage of males in the population in Lakes Superior and Huron. For example, at the peak of their abundance in 1960-1962 the adult population was roughly 70% male (Smith et al. 1974). Once their numbers began to drop as a result of the treatment program, the percentage of males in the population decreased significantly, until between 1971 and 1978 the males made up only about 30-40% (Heinrich et al. 1980). This apparent shift to "femaleness" in the adults of the upper Great Lakes is reflected by a corresponding change in larval populations (Torblaa and Westman 1980). The mechanism behind this changeover remains unclear. Smith (1971) speculated that a shift in sex ratio towards males at high population levels and toward females at low levels might be a natural means of population control. However, between 1968 and 1977 in Lake Ontario, the percentage of adult males has remained stable at 50-58% of the population (Torblaa and Westman 1980). The sex ratio of larval populations has been observed to vary with different physicochemical conditions, suggesting that environmental factors, e.g. temperature and density, may also play a role in determining the sexual differentiation of the ammocoetes.

8.3.6 EFFECTS ON SPAWNING RUNS

Moore and Schleen (1980) observed that following the successful treatment of a stream, a dramatic decline can occur in the number of adults that enter the stream and spawn the following year. This effect was particularly noticeable in streams which were treated late in the year and which contained no known lentic populations of larval sea lampreys. Part of the reason for this observation may be related to the attraction between sea lampreys. Teeter (1980) found that sexually immature male sea lampreys captured during a spawning migration displayed a statistically significant preference for water from tanks which contained sea lamprey ammocoetes, and that this preference rose as the number of ammocoetes increased. For example, in a tank with a sand substrate containing two compartments, one of which held 10 ammocoetes, sexually immature sea lampreys spent about 70% of their exposure time in the compartment containing the ammocoetes. Doubling the number of larvae increased the response to about 80%. Similar preferences were noted when 4 L of water from a 38-L tank, which had held 36 ammocoetes for either 24 or 144 h, was used as a stimulus.



9.0 EFFECTS OF LAMPRICIDES ON AQUATIC MACROPHYTES AND ALGAE

Information on the effects of Bayer 73 on aquatic plants and algae is fragmentary and largely qualitative in nature, and is therefore of little use in evaluating possible effects in the field. The toxicity of the 98:2 mixture to algae and aquatic vegetation has not been investigated. Consequently, only the effects of TFM will be discussed in this section.

9.1 EFFECTS OF TFM ON AQUATIC MACROPHYTES

At one time, various salts of TFM were patented for use as aquatic herbicides (Josephs 1961 as cited in Gilderhus and Johnson 1980). TFM controlled aquatic plants such as Anacharis sp., Cabomba sp. and Ceratophyllum sp. at concentrations of 15-25 mg.L⁻¹ in standing water and 100 mg.L⁻¹ in flowing water, in exposure periods of 2 h (Schnick 1972). However, these concentrations are much higher than those used in sea lamprey treatments (Maki and Johnson 1977a; Gilderhus and Johnson 1980).

In a recirculating stream system, Maki and Johnson (1977a) showed that Canadian pondweed (Elodea canadensis) exposed to TFM concentrations of 5, 10, 15, 20 and 35 mg.L⁻¹ over 24 h, suffered a loss in weight at all levels greater than 5 mg.L⁻¹. Exposure for periods longer than 1 h significantly reduced the production of plants subjected to concentrations ≥ 10 mg.L⁻¹ TFM. In addition, those plants exposed to 20 and 35 mg.L⁻¹ TFM for longer than 3 h became limp and cyanotic. Nevertheless, plants subjected to concentrations up to a maximum of 20 mg.L⁻¹ had the potential to recover given sufficient time and suitable growth conditions. In contrast, individual plants exposed to 35 mg.L⁻¹ TFM for periods longer than 1 h, died. Similar experiments indicated that European milfoil (Myriophyllum spicatum) was slightly more susceptible to TFM. Although great variability in growth rate was noted, reductions in biomass of 60-85% of that of the controls at concentrations of 10-25 mg.L⁻¹ TFM did occur. However, at the concentrations and times of exposure used for lamprey control in water conditions comparable to those used in their experiments (6-7 mg.L⁻¹ for 10 h), Maki and Johnson (1977a) concluded that in the 2-week period following a treatment, only a slight reduction in growth rate would occur, amounting to 5-10% for E. canadensis and perhaps 20% for M. spicatum.

9.2 EFFECTS OF TFM ON ALGAE

Maki et al. (1975a) exposed 10 species of algae to TFM for 96 h and demonstrated that all species, except Chlorella pyrenoidosa, suffered a 50% inhibition of growth at concentrations less than 10 mg.L⁻¹ (Table 9-1). Chlorella pyrenoidosa was the most resistant

Table 9-1. Concentrations of formulated TFM^a causing a 50% growth inhibition in 10 different species of algae (from Maki et al. 1975a).

Species	96-h IC ₅₀ ^b (mg.L ⁻¹)	95% confidence interval
Green		
<u>Scenedesmus quadricauda</u>	4.2	2.21-7.98
<u>Stigeoclonium tenue</u>	4.7	3.24-6.81
<u>Selenastrum capricornutum</u>	5.5	4.14-7.32
<u>Chlorella pyrenoidosa</u>	>15	-
Blue-green		
<u>Cylindrospermum</u> sp.	9.0	7.50-10.8
<u>Anabaena flos-aquae</u>	3.6	2.40-5.40
<u>Nostoc linckia</u>	9.2	8.00-10.6
<u>Anabaena cylindrica</u>	1.8	0.82-3.96
Diatoms		
<u>Nitzschia</u> sp.	1.2	0.71-2.04
<u>Navicula pelliculosa</u>	3.2	2.00-5.12

^a TFM is a 35.7% by weight active ingredient formulation.

^b 96-h IC₅₀ is the concentration producing a 50% inhibition of growth in each species of algae in 96 hours.

species, having a 96-h IC_{50} of $> 15 \text{ mg.L}^{-1}$. However, concentrations as high as 30 mg.L^{-1} only arrested growth, but did not kill the algae. After they were resuspended in a TFM-free medium the algae resumed growth, although several days were required for the cell density to approach that of the controls. Despite considerable variation among species, it appeared that based on mean 96-h IC_{50} values, the blue-green algae were the most resistant (7.9 mg.L^{-1}), green algae were intermediate (5.8 mg.L^{-1}) and diatoms were most sensitive (2.2 mg.L^{-1}) (Maki 1980).

In a model stream study, TFM depressed the production of oxygen by the algal community and increased the respiration rate (Maki and Johnson 1976b). During the summer when the filamentous green algae were dominant, TFM reduced the gross production rate only 5-10%, whereas in the fall when the more sensitive diatoms were dominant, the depression was much greater (25-50%). This effect was only temporary, because gross production returned to pretreatment rates within 24 to 36h.

Maki (1980) examined the standing crop of filamentous green algae in model streams from early summer to the beginning of winter. He observed that a gradual succession of species occurred throughout the year, with a peak biomass in August slowly declining until December. Exposure of the experimental stream to a concentration of 9 mg.L^{-1} TFM for 24 h had no effect on the relative frequency of occurrence of different species or on the biomass of the standing crop. Even exposure to 30 mg.L^{-1} TFM for 4 days did not decrease the viability of algal cells when they were resuspended in TFM-free water. Hence, it appears that TFM has an algistatic rather than an algicidal effect and that this effect is only temporary.



10.0

EFFECTS OF LAMPRICIDES ON INVERTEBRATES

10.1 EFFECTS OF TFM

The acute toxicity of TFM to stream invertebrates has been determined largely in the laboratory (Smith 1967a; Chandler and Marking 1975; Fremling 1975; Maki et al. 1975b; Sanders and Walsh 1975; NFRL 1982a). The effects of TFM in the field were reported in a study by Torblaa (1968) and as observations made by treatment crews (Gilderhus and Johnson 1980).

One of the problems encountered in conducting acute studies with invertebrates is determining whether the toxicant has killed or merely temporarily immobilized the organism (Kawatski et al. 1975; Hansen and Kawatski 1976). Smith (1967a) first noted this effect in leeches, snails and clams, which appeared "sluggish" after testing. Hansen and Kawatski (1976) found that the aquatic midge, Chironomus tentans, had a 24-h LC_{50} more than 6 times greater than the 24-h EC_{50} (time to immobilization). However, an organism that becomes immobilized may be more susceptible to predation.

Maki et al. (1975b) approximated natural conditions by exposing aquatic worms, crustaceans, insects (nymphs and larvae), and molluscs to TFM in model stream systems and verified whether any apparent mortality had actually occurred, by placing affected individuals in TFM-free water after treatment. The results are given in Table 10-1.

Aquatic annelids were among the most sensitive organisms exposed to TFM. This may be related to their high uptake and limited clearance capacity (see Section 5.2.2.1). In the study by Maki et al. (1975b), aquatic earthworms had one of the lowest 24-h LC_{50} s, 6.6 $mg.L^{-1}$. Chandler and Marking (1975) found that in water with a hardness of 20 $mg.L^{-1}$ $CaCO_3$ and pH 6.8-7.0, Tubifex tubifex was very sensitive, with a 24-h LC_{50} of 3.43 $mg.L^{-1}$. Smith (1967a) predicted on the basis of a lab study, that all turbellarians would be eliminated at concentrations sufficient to kill sea lamprey ammocoetes. In field applications, dead aquatic earthworms have been observed in many instances (U.S. Bureau Commercial Fisheries 1958, 1959, 1960, 1961). Similarly, Gilderhus et al. (1975) found that annelids were eliminated from a stream following a 12-h treatment with 11 $mg.L^{-1}$ TFM.

In contrast, crustaceans were largely unaffected by TFM in tests by Smith (1967a), Chandler and Marking (1975), Maki et al. (1975b) and Sanders and Walsh (1975). Among the most resistant species were the scud, Gammarus pseudolimnaeus, and the crayfish, Orconectes propinquus; they had 24-h LC_{50} values of 38.0 and > 36.4 $mg.L^{-1}$ TFM respectively. Similarly, Kawatski (1973) determined that the ostracod, Cypretta kawatai, was tolerant as it had a 24-h LC_{50} of 14.2 $mg.L^{-1}$ TFM in soft water (40-48 $mg.L^{-1}$ $CaCO_3$).

Table 10-1. Toxicity of formulated TFM^a to aquatic macroinvertebrates. All insect nymphs and larvae are late instars (hardness = 211 mg.L⁻¹ CaCO₃, pH = 7.79, temperature = 12.0°C) (adapted from Maki et al. 1975b).

Organism	Size (mm)	24-h LC ₅₀ (mg.L ⁻¹)	95% confidence interval
Oligochaetes			
Annelida			
Lumbricidae	6-9	6.6	6.11-7.13
Crustaceans			
Isopoda			
<u>Asellus militaris</u> ^β	8-10	17.0	14.41-20.06
Amphipoda			
<u>Gammarus pseudolimnaeus</u>	10-12	38.0	32.76-44.08
Decapoda			
<u>Orconectes propinquus</u>	3-4	>36.4	
Aquatic insects			
Plecoptera			
<u>Isoperla slossonae</u>	-	16.7	13.47-20.71
<u>Paragnetina media</u>	-	21.5	17.92-25.81
<u>Pteronarcys dorsata</u>	-	32.2	23.85-43.47
<u>Acroneura lycorias</u>	-	>34.0	-
Odonata			
<u>Ophiogomphus</u> sp.	-	>38.0	-
Ephemeroptera			
<u>Baetis</u> sp.	-	6.9	6.39-7.45
<u>Hexagenia bilineata</u>	-	7.9	6.93-9.01
<u>Cloeon</u> sp.	-	11.1	8.88-13.87
<u>Isonychia bicolor</u>	-	18.3	15.77-21.23
<u>Stenonema frontale</u>	-	24.9	22.23-27.89
<u>Tricorythodes</u> sp.	-	29.3	25.86-33.19

Table 10-1. (Cont'd)

Organism	Size (mm)	24-h LC ₅₀ (mg.L ⁻¹)	95% confidence interval
<u>Stenonema luteum</u>	-	29.6	27.92-31.37
<u>Baetisca obesa</u>	-	37.8	33.16-43.09
<u>Paraleptophlebia</u> sp.	-	>34.6	-
<u>Ephemerella cornuta</u>	8	>39.0	
Megaloptera			
<u>Cauliodes</u> sp.	-	>36.0	-
Trichoptera			
<u>Chimarra obscura</u>	-	3.8	3.59-4.04
<u>Brachycentrus americanus</u>	-	10.5	8.40-13.13
<u>Lepidostoma</u> sp.	-	15.0	13.64-16.50
<u>Cheumatopsyche</u> sp.	-	28.3	24.71-32.40
<u>Hydropsyche</u> sp.	-	>32.0	-
<u>Macrostenum</u> sp.	-	>38.0	-
<u>Limnephilus consocius</u>	-	>39.0	-
Diptera			
<u>Simulium pugetense</u>	-	6.1	5.65-6.59
Molluscs			
Pelecypoda			
<u>Ligumia</u> sp.	<90	>11.2	-
<u>Ligumia</u> sp.	>160	>18.0	-
<u>Pisidium</u> sp.	10-12	16.9	14.93-19.13
<u>Sphaerium</u> sp.	10-15	17.5	16.84-18.23

^a TFM is a 36% by weight active ingredient formulation.

^b The exact identity of this organism cannot be determined.

Considerable variability in sensitivity to TFM exists among aquatic insect orders. Among the Diptera, only blackfly larvae (Simuliidae) appear to be susceptible (Smith 1967a; Maki *et al.* 1975b). Snipe flies (Rhagionidae) and chironomids (Chironomidae) were resistant, having 24-h LC_{50} s of $> 20 \text{ mg.L}^{-1}$ (Smith 1967a).

Among the Ephemeroptera (mayflies), only three genera were found to be especially sensitive to TFM, *Baetis* sp., *Hexagenia* sp. and *Cloeon* sp. (Maki *et al.* 1975b). All of the others listed in Table 10-1 were relatively resistant; their 24-h LC_{50} values ranged from 18 to 40 mg.L^{-1} . However, field studies have indicated that certain mayflies, especially *Hexagenia* sp., have been significantly affected by TFM treatments. Applegate *et al.* (1961) noted that TFM seemed to cause the early emergence of mayflies, an observation later confirmed by Maki *et al.* (1975b) in the laboratory. Torblaa (1968) found reduced numbers of mayflies one week after a stream had been treated with TFM. In addition, treatment crews reported seeing dead mayflies more often than any other invertebrates (Gilderhus and Johnson 1980). A more detailed study of the toxicity of TFM to several stages of development of the mayfly genus, *Hexagenia* sp., known to be present during stream treatments, has determined that as mayflies develop from embryos to 27-mm nymphs, they become more sensitive (NFRL 1982a). In soft water (hardness = $44 \text{ mg.L}^{-1} \text{ CaCO}_3$) at 17°C , a 24-h LC_{50} was established at 1.5 mg.L^{-1} for 23-mm nymphs. This finding is in close agreement with the results of Fremling (1975), who determined a value of 1.70 mg.L^{-1} for nymphs, 20-22 mm in length. It was also found that swimming nymphs were twice as susceptible as burrowed individuals, a pattern similar to that observed for sea lamprey ammocoetes.

The sensitivity of caddisflies to TFM (Order Trichoptera) is also variable both in the laboratory (Table 10-1) and in the field (Torblaa 1968). *Chimarra obscura* was the most sensitive organism tested (24-h $LC_{50} = 3.8 \text{ mg.L}^{-1}$ TFM) (Table 10-1). Other genera were more resistant, having LC_{50} values from 10.5 to $>39.0 \text{ mg.L}^{-1}$. Smith (1967a) also observed different sensitivities in caddisflies and noted that case-building species were more resistant than net-builders.

The stoneflies (Plecoptera) tested by Maki *et al.* (1975b) were all relatively resistant to TFM. They had 24-h LC_{50} values ranging from 16.7 to $>34.0 \text{ mg.L}^{-1}$. The dobsonflies (Megaloptera) and dragonflies (Odonata) were very resistant to TFM, having 24-h LC_{50} s of >36.0 and $>38.0 \text{ mg.L}^{-1}$ respectively.

The pelycypod molluscs showed an intermediate sensitivity with 24-h LC_{50} values varying from > 11.2 to $>18.0 \text{ mg.L}^{-1}$. In the field, however, clams have been reported as being susceptible (U.S. Bureau Commercial Fisheries 1960, 1961).

Obviously a large degree of variation in sensitivity to TFM exists among and within groups. For instance, the 24-h LC_{50} for aquatic

annelids was only 6.6 mg.L⁻¹ TFM as opposed to 36.4 mg.L⁻¹ for the crustacean, Orconectes propinquus. Within a single group the greatest difference was observed among the Trichoptera, where the 24-h LC₅₀ of Limnephilus consocius was more than 10 times that of Chimarra obscura.

Maki et al. (1975b) calculated that based on the water hardness (211 mg.L⁻¹ as CaCO₃) and pH (7.79) of the model streams, a concentration of 9 mg.L⁻¹ TFM would be required to eliminate sea lamprey larvae. Using that figure as a basis of comparison, it was estimated that populations of aquatic annelids and larval blackflies would be reduced to less than 50% of their pretreatment abundance. Three genera of mayflies (Hexagenia sp., Baetis sp. and Cloeon sp.) would suffer heavy mortalities and the caddisfly, Chimarra obscura, would be eliminated. For the remaining species, predicted effects were considered to be minimal since the 24-h LC₅₀ values were roughly 1.5 to 4 times greater than the treatment concentration.

Maki (1980) determined that the species diversity indices (Shannon-Weaver) in pool and riffle communities, before and after a TFM treatment of natural and model stream systems, were not significantly different. The most apparent effect of a TFM treatment was an immediate reduction in macroinvertebrate density. Although it was not statistically significant, the total number of individuals per unit area dropped to about 20% of the pretreatment abundance one day after exposure. Similarly, Torblaa (1968) reported that the percentage of groups (annelids, crustaceans, aquatic insect larvae and molluscs) exhibiting a decline in numbers in detrital and riffle areas was 77% and 64%, respectively. However, this effect was apparently only temporary. Maki (1980) observed a rapid recolonization 4 weeks after exposure, while Torblaa (1968) noted a rapid recovery in 4 out of 5 treated streams within 6 weeks. One year later, Torblaa (1968) sampled 3 of the treated streams and found an apparent recovery with regard to both the numbers of organisms and major taxonomic groups present.

One of the most striking effects observed by Maki (1980) was a pulse in the number of drifting organisms immediately after the introduction of TFM. The pretreatment drift rate in both the control and experimental channels was 1-5 individuals per sampling period. Immediately after the introduction of TFM, the drift rate increased dramatically to a peak of 32 individuals per sampling period, before it stabilized within 3 h at about twice the control rate. Maki (1980) suggested that the increased drift pulse, together with the mortalities of susceptible species, was largely responsible for the decline in macroinvertebrate density after an application of TFM. This observed rise in drift numbers was largely due to only a few species; a mayfly (Baetis sp.), a caddisfly (Dolophilodes distinctus) and an amphipod (Gammarus pseudolimnaeus).

The results of a recent field study (Kolton et al. 1985) concerning the effects of TFM on benthic invertebrates following an

actual TFM treatment are consistent with those presented above. A significant decline in relative abundance of the 68 taxa examined during the 19-day period following treatment was noted for five groups only: Dugesia sp. (turbellarian), Lumbricidae, Tubificidae, Caenis sp. (nonburrowing mayfly) and Chimarra sp. Two days after the application of TFM, the relative abundance of Dugesia sp. and Tubificidae declined 90% and 55% respectively. After 19 days, the Lumbricidae and Chimarra sp. were virtually eliminated. Similarly 3 weeks after the treatment of a tributary of Lake Superior with TFM, Dermott and Spence (1984) found that philopotamid caddisflies (Dolophilodes sp. and Chimarra sp.) had been eliminated while the abundance of Lumbricidae (Stylodrilus sp.) had been reduced by 70%.

Kolton et al. (1985) noted that the rate of drift increased significantly for Dugesia sp., Tubificidae, Erpobdella sp. (annelid), Chimarra sp. and Homerodromia sp. (Diptera) within 4.5 h after the initiation of treatment. For example, Dugesia sp. and Tubificidae together represented less than 18% of the invertebrates collected in pretreatment drift samples; yet during the treatment they accounted for 290%. With the exception of Chimarra sp., the drift pulse declined to pretreatment levels 8 h after the treatment ended. The drift abundance of Chimarra sp. was still above treatment levels when the sampling stopped 48 h later.

Seven months after the treatment, further sampling indicated that the adversely affected taxa had recovered. The mechanism of recolonization was attributed to upward migration from within the hyporheos.

In the field, the scud, Gammarus sp., has repeatedly been reported as having been killed by TFM (U.S. Bureau Commercial Fisheries 1958, 1959, 1960, 1961). Yet, considering the degree of resistance of Gammarus pseudolimnaeus (Table 10-1), and the observed numbers in the drift pulse (Maki 1980), it is likely that what were thought to be dead organisms, were actually scud drifting downstream. Hyperactivity in arthropods has been induced by TFM (Maki et al. 1975b).

In addition to causing hyperactivity in arthropods and molluscs and the early emergence of insects, TFM also has been shown to increase the basal metabolic rate of the midge, Chironomus tentans (Kawatski et al. 1974). Although the exact significance of this observation is unclear, it may be related to the mode of action of TFM (see Section 6.1).

10.2 EFFECTS OF A 98:2 MIXTURE OF TFM AND BAYER 73

The acute toxicity of a 98:2 mixture of TFM and Bayer 73 to aquatic invertebrates is based on a laboratory study by Rye and King (1976), the results of which are given in Table 10-2. Data from the

Table 10-2. Toxicity of a 98:2^a mixture of TFM and Bayer 73 and of Bayer 73^b alone to aquatic invertebrates^Y (hardness = 100-136 mg.L⁻¹ CaCO₃, pH = 8.2-8.3, temperature = 12.8°C) (adapted from Rye and King 1976).

Organism	24-h LC ₅₀ and 95% confidence interval (mg.L ⁻¹)	
	98:2 mixture	Bayer 73
Turbellaria		
<u>Dugesia tigrina</u>	1.5 (1.42-1.58)	0.048 (0.044-0.053)
Oligochaetes		
Annelida		
<u>Tubifex tubifex</u>	2.25 (1.98-2.55)	0.034 (0.031-0.037)
<u>Lumbriculus inconstans</u>	5.2 (4.8-5.6)	0.14 (0.12-0.18)
Hirudinea		
Erpobdellidae	4.0 (3.3-4.9)	0.42 (0.38-0.47)
Crustaceans		
Cladocera		
<u>Daphnia pulex</u>	7.35 (5.47-9.41)	0.8 (0.68-0.94)
Isopoda		
<u>Asellus militaris</u> ⁶	31.0 (28.7-33.5)	23.0 (18.4-28.8)
Amphipoda		
<u>Gammarus</u>	26.0 (24.4-27.7)	2.6 (2.34-2.88)
Decapoda		
<u>Orconectes</u> sp.	>100.0 -	>50.0 -
Aquatic insects ^Y		
Plecoptera		
<u>Paragnetina</u> sp.	15.4 (12.4-19.0)	1.07 (0.79-1.44)
Odonata		
<u>Ophiogomphus</u> sp.	>100.0 -	>50.0 -
Ephemeroptera		
<u>Hexagenia</u> sp.	4.0 (3.6-4.5)	6.9 (5.85-8.14)

Table 10-2. (Cont'd)

Organism	24-h LC ₅₀ and 95% confidence interval (mg.L ⁻¹)	
	98:2 mixture	Bayer 73
<u>Stenonema</u> sp.	30.5 (26.1-35.7)	2.27 (1.68-3.08)
Megaloptera <u>Corydalis</u> sp.	>100.0 -	>50.0 -
Trichoptera <u>Hydropsyche</u> sp.	30.0 (25.6-35.1)	2.45 (1.88-3.19)
<u>Helicopsyche</u> sp.	48.0 (38.1-60.5)	1.67 (1.18-2.37)
Diptera <u>Simulium</u> sp.	3.45 (3.05-3.89)	0.255 (0.236-0.275)
<u>Atherix</u> sp.	>100.0 -	>50 -
Molluscs Gastropoda <u>Physa</u> sp.	1.93 (1.54-2.41)	0.106 (0.097-0.116)
<u>Pleurocerca</u> sp.	9.4 (7.74-11.42)	0.355 (0.290-0.430)
Pelecypoda <u>Elliptio dilatatus</u>	4.7 (3.5-6.3)	0.382 (0.320-0.458)

^a 98:2 is a mixture of TFM and Bayer 73 in the proportion 98:2 by weight based on active ingredient.

^b Bayer 73 is a 70% by weight active ingredient formulation.

^c All aquatic insects were exposed as nymphs or larvae while all other invertebrates were exposed as adults.

^d The exact identity of this organism cannot be determined.

field are derived from studies by Torblaa (1968) and Dermott and Spence (1984).

Although the data in Table 10-2 cannot be directly compared with those for TFM in Table 10-1 because of the difference in water hardness, similar trends in toxicity are evident. The turbellarians, oligochaetes and leeches are all relatively susceptible; their 24-h LC_{50} s range from 1.5 to 5.2 $mg.L^{-1}$ of the 98:2 mixture.

Among crustaceans, isopods, amphipods and decapods all were resistant (Table 10-2). Similarly, the ostracod, Cyprretta kawatai, was not affected (24-h LC_{50} = 13.9 $mg.L^{-1}$) (Kawatski 1973). The daphnids were more susceptible; the 24-h LC_{50} was only 7.35 $mg.L^{-1}$.

Aquatic insects exhibit great variability in their sensitivity to the 98:2 mixture. For example, among Diptera, blackfly larvae, Simulium sp., were susceptible with a 24-h LC_{50} of only 3.45 $mg.L^{-1}$ whereas snipeflies, Atherix sp., were extremely resistant, having an LC_{50} of >100 $mg.L^{-1}$. Similarly, in the Ephemeroptera, Hexagenia sp. was sensitive while Stenonema sp. was more than seven times as resistant. The 98:2 mixture and TFM were equally toxic to 23-mm nymphs of the mayfly Hexagenia (NFRL 1982a). In addition, the exposure of different lifestages to the 98:2 mixture resulted in similar responses to those observed with TFM, i.e. the embryo was the most resistant, followed by increasing susceptibility as development progressed.

It appears that the Trichoptera also exhibit variability in their response to the mixture for, on the one hand, the data in Table 10-2 suggest a marked resistance, and on the other, Torblaa (1968) noted that the Psychomyiidae suffered a 94% reduction in numbers in a stream treated with the 98:2 mixture. Based on the results of tests on single species (Table 10-2), the Plecoptera, Megaloptera and Odonata were all tolerant of the mixture.

The sensitivity of molluscs to the mixture varied considerably. For instance, among snails, Physa sp. was susceptible (24-h LC_{50} = 1.93 $mg.L^{-1}$), whereas Pleurocera sp. was almost five times as resistant (24-h LC_{50} = 9.4 $mg.L^{-1}$). The clam, Elliptio dilatatus, showed an intermediate tolerance.

The results of a field study by Dermott and Spence (1984) using a 99.2:0.8 mixture of TFM and Bayer 73 generally support the findings of the laboratory work by Rye and King (1976). Significant declines in biomass four days after treatment were reported for turbellarians, leeches (Piscicolidae) and oligochaetes (mainly Stylodrilus heringianus and Limnodrilus hoffmeisteri). The latter group experienced a decline from 53% of the total dry biomass to only 12% following treatment.

Daytime drift rates of Turbellaria, Annelida, (especially Piscicolidae), Amphipoda, Glossosomatidae and Tipulidae were increased

significantly during treatment. Leeches were affected most, as their biomass (dry weight) in the drift increased from 0.005 to 2.61 g.1000m⁻³. Dermott and Spence (1984) interpreted the results to be an indication that increased drift, rather than direct mortality, was the major impact of lampricides on invertebrate communities.

10.3 EFFECTS OF BAYER 73

Our knowledge of the acute toxicity of Bayer 73 to aquatic invertebrates is based on laboratory studies by Rye and King (1976) and Sanders (1977) and a field study by Gilderhus (1979). Using Table 10-2, the comparative toxicity of Bayer 73 to several invertebrates can be determined from the 24-h LC₅₀ values listed. However, it is difficult to assess what effects would occur in the field from the use of 5% granular Bayer 73, because the treatments are designed to produce a high concentration of Bayer 73 in a limited bottom area, over a short period of time (see Section 3.1.1). For example, in Canada, a theoretical concentration of 23 mg.L⁻¹ in the bottom 5 cm of water would remain active against larval lampreys for less than 2 h (Gilderhus and Johnson 1980). Hence, in this section, the sensitivity of invertebrates will be discussed on the basis of observed 24-h LC₅₀ values and observations from the field.

Bayer 73 is much more toxic to most invertebrates than TFM or a 98:2 mixture (Table 10-2 and 10-1). Some of the most susceptible organisms are turbellarians, oligochaetes and leeches, since all have 24-h LC₅₀s < 0.50 mg.L⁻¹. The results of a field study by Gilderhus (1979) confirmed that oligochaetes have a low tolerance to Bayer 73. One day after treatment, the population had declined sharply, and after seven days it remained at only 20% of its pretreatment abundance. Although this reduction was not statistically significant because of the high variability among samples, there was a strong trend that suggested that oligochaetes would be severely reduced by Bayer 73. The effect on leeches could not be determined because of sampling variation.

Crustaceans show a mixed response to Bayer 73. The cladoceran, Daphnia pulex, exhibited extreme sensitivity (24-h LC₅₀ of only 0.8 mg.L⁻¹). Shiff and Garnett (1961) found that microcrustaceans such as cladocera, copepods and ostracods were markedly reduced in ponds immediately after treatments with 1 mg.L⁻¹ Bayer 73. But populations returned to pretreatment levels after 32 days. The amphipod, Gammarus sp., appeared to be relatively susceptible according to Rye and King (1976), but was apparently unaffected in a field treatment (Gilderhus 1979). The isopod, Asellus militaris^a, (LC₅₀ =

^a The exact identity of this organism cannot be determined.

23.0 mg.L⁻¹) appeared to be resistant to Bayer 73. Similarly, crayfish displayed great resistance, with LC₅₀ values ranging from 32.0 (Sanders 1977) to >50 mg.L⁻¹ (Rye and King 1976).

Aquatic insects display a wide range of sensitivities in response to Bayer 73, as was observed with TFM and the 98:2 mixture. Among the Order Diptera, the blackfly, Simulium sp., was very sensitive (Table 10-2). Kawatski et al. (1975) found that the 24-h EC₅₀ (immobilization) of the midge, Chironomus tentans, was 0.57 mg.L⁻¹. Gilderhus (1979) also found that the midge was sensitive to Bayer 73, as evidenced by a 54% decline in the population by day 7 posttreatment. In contrast, the snipefly larvae were very resistant (24-h LC₅₀ >50 mg.L⁻¹).

Among the Ephemeroptera, Hexagenia sp. was moderately resistant to Bayer 73, in contrast to its pronounced susceptibility to TFM and the 98:2 mixture (Table 10-2). It was also noted that 25-mm nymphs were resistant to concentrations as high as 10 mg.L⁻¹ for 24 h and that free-swimming individuals were as resistant as burrowed ones (NFRL 1982b). The other genus tested, Stenonema sp., was three times more susceptible. In the field, Gilderhus (1979) determined that Bayer 73 had no significant effect on mayfly populations.

Caddisflies appeared to be relatively susceptible to Bayer 73 with 24-h LC₅₀s ≤ 2.45 mg.L⁻¹. This was confirmed in the field where 13 days after treatment, the caddisfly population was observed to have been eliminated (Gilderhus 1979). Similarly, in an unpublished study cited by Gilderhus and Johnson (1980), the caddisfly population was reduced by about 90% by Bayer 73. Stonefly nymphs also appear susceptible to Bayer 73, but they are unlikely inhabitants of lentic areas (Gilderhus and Johnson 1980). Other Orders such as Megaloptera and Odonata were very resistant (24-h LC₅₀s > 50 mg.L⁻¹; see Table 10-2).

The molluscs tested (Table 10-2) all showed extreme sensitivity to Bayer 73 (24-h LC₅₀ values < 0.4 mg.L⁻¹). This would be expected since Bayer 73 was originally developed as a molluscicide to eliminate snails, which are intermediate hosts of the parasite causing schistosomiasis in man (Gönnert 1962). Unfortunately, in the study of Gilderhus (1979) the numbers of snails present were too low and too variable to permit an assessment of the effect of Bayer 73. However, it was established that a population of clams was eliminated 13 days after treatment.



11.0 EFFECTS OF LAMPRICIDES ON NONTARGET FISH

11.1 ACUTE TOXICITY OF TFM AND THE 98:2 MIXTURE

11.1.1 LABORATORY STUDIES

Preliminary results of the acute toxicity of TFM to nontarget fish indicated that they could be grouped into three general classes of sensitivity; very resistant, resistant and susceptible (Applegate *et al.* 1961; Applegate and King 1962). The most resistant group was the centrarchids, e.g. bluegill (Lepomis macrochirus), smallmouth bass (Micropterus dolomieu) and largemouth bass (Micropterus salmoides), whereas the most susceptible group included the bullheads (Ictalurus spp.), logperch (Percina caprodes), white sucker (Catostomus commersoni), walleye (Stizostedion vitreum vitreum) and yellow perch (Perca flavescens). Species intermediate in sensitivity were the salmonids, e.g. rainbow trout, brook trout (Salvelinus fontinalis) and brown trout (Salmo trutta), and the cyprinids, e.g. fathead minnow (Pimephales promelas), blacknose shiner (Notropis heterolepis) and golden shiner (Notemigonus crysoleucas).

With the exception of yellow perch and walleyes (which exhibit an intermediate sensitivity) recent studies have generally supported these findings (Marking and Olson 1975; Marking *et al.* 1975; Bills and Marking 1976). From Table 11-1, based on a decreasing order of resistance according to the 24-h LC_{50} values, the bluegill and green sunfish, Lepomis cyanellus, were found to be the most resistant species tested, with 24-h LC_{50} s of 7.05 and 5.62 $mg.L^{-1}$ respectively. In contrast, the black bullhead (Ictalurus melas) and channel catfish (Ictalurus punctatus) were the most susceptible, with respective LC_{50} s of 1.09 and 1.05 $mg.L^{-1}$. Other species were intermediate in sensitivity; their 24-h LC_{50} s ranged from 1.46 to 3.57 $mg.L^{-1}$. Using analytical grade TFM, Marking and Olson (1975) found that the 96-h LC_{50} of northern pike, Esox lucius, was not significantly different from that of channel catfish. The data of Bills and Marking (1976), based on 96-h LC_{50} s, follow a pattern similar to that of Marking and Olson (1975) (Table 11-1).

It should also be noted that all species listed in Table 11-1 reacted rapidly to TFM, as indicated by the 1-h LC_{50} s, which were only 2-5 times higher than the 24-h LC_{50} s. In most cases there was little or no change in toxicity between 24 and 96 h. Marking *et al.* (1975) observed the same effect in coho salmon (Oncorhynchus kisutch), rainbow trout, brown trout, brook trout, lake trout and bluegill. In this study, the LC_{50} did not change significantly between 6 and 96 h in hard water (300 $mg.L^{-1}$ $CaCO_3$).

Lethal effects of a 98:2 mixture on nontarget fingerling fish were reported by Bills and Marking (1976), based on a 96-h LC_{50} laboratory study (Table 11-2). The general pattern observed for TFM also

Table 11-1. Toxicity of formulated TFM^a to fingerling fish (1-1.5 g)
(temperature 12°C, hardness = 40-48 mg.L⁻¹, pH = 7.2-7.6)
(adapted from Marking and Olson 1975).

Species	LC ₅₀ and 95% confidence interval (mg.L ⁻¹)			
	1 h	24 h	96 h	96 h ^b
Bluegill sunfish (<i>Lepomis macrochirus</i>)	-	7.05 (5.92-8.40)	7.05 (5.92-8.40)	4.89 (4.27-5.60)
Green sunfish (<i>Lepomis cyanellus</i>)	11.40 (10.58-12.30)	5.62 (4.97-6.36)	4.10 (3.43-4.87)	-
Golden shiner (<i>Notemigonus crysoleucas</i>)	8.19 (7.97-8.40)	3.57 (3.09-4.13)	3.32 (2.74-4.02)	-
Largemouth bass (<i>Micropterus salmoides</i>)	-	2.63 (1.77-3.91)	2.63 (1.77-3.91)	-
Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	5.06 (4.24-6.01)	2.62 (2.24-3.09)	1.85 (1.55-2.20)	-
Yellow perch (<i>Perca flavescens</i>)	4.97 (4.35-5.62)	2.52 (2.16-2.94)	1.89 (1.50-2.39)	1.71 (1.47-1.98)
Fathead minnow (<i>Pimephales promelas</i>)	7.63 (6.92-8.40)	2.09 (1.82-2.39)	2.09 (1.82-2.39)	-
Brown trout (<i>Salmo trutta</i>)	4.23 (3.71-4.83)	1.99 (1.70-2.34)	1.55 (1.34-1.80)	0.94 (0.79-1.17)
White sucker (<i>Catostomus commersoni</i>)	4.35 (3.59-5.26)	1.96 (1.41-2.73)	1.72 (1.17-2.53)	-
Rainbow trout (<i>Salmo gairdneri</i>)	2.56 (2.35-2.78)	1.69 (1.45-1.94)	1.69 (1.45-1.94)	1.81 (1.53-2.14)
Lake trout (<i>Salvelinus namaycush</i>)	6.37 (5.49-7.42)	1.69 (1.37-2.08)	1.29 (1.16-1.44)	1.78 (1.51-2.10)
Carp (<i>Cyprinus carpio</i>)	3.60 (3.05-4.26)	1.46 (1.03-2.07)	1.46 (1.03-2.07)	-
Black bullhead (<i>Ictalurus melas</i>)	5.88 (5.31-6.48)	1.09 (0.82-1.27)	1.05 (0.91-1.21)	-
Channel catfish (<i>Ictalurus punctatus</i>)	5.19 (4.44-6.05)	1.05 (0.91-1.21)	0.60 (0.49-0.75)	0.75 (0.62-0.91)

^a TFM is a 35.7% by weight active ingredient formulation for chinook salmon, brown trout, lake trout and rainbow trout and 35.4% for all other species. Data were given as µL.L⁻¹. Specific gravity was taken into account by multiplying by a factor of 1.23.

^b From Bills and Marking (1976) using 0.5-1.5 g fish and a 35.7% by weight active ingredient formulation of TFM.

Table 11-2. Toxicity of a 98:2 mixture^a of TFM and Bayer 73 to fingerling fish (0.5-1.5 g) (temperature = 12°C, hardness = 44 mg.L⁻¹, pH = 7.5) (from Bills and Marking 1976).

Species	96-h LC ₅₀ and 95% confidence interval (mg.L ⁻¹)	
	TFM	Bayer 73
Bluegill (<u>Lepomis macrochirus</u>)	3.18 (2.57-3.94)	0.0636 (0.0514-0.0788)
Lake trout (<u>Salvelinus namaycush</u>)	1.39 (1.12-1.72)	0.0284 (0.0229-0.0351)
Rainbow trout (<u>Salmo gairdneri</u>)	1.16 (0.998-1.35)	0.0237 (0.0204-0.0275)
Brook trout (<u>Salvelinus fontinalis</u>)	1.16 (0.786-1.71)	0.0237 (0.0160-0.0349)
Brown trout (<u>Salmo trutta</u>)	0.98 (0.810-1.19)	0.0200 (0.0165-0.0242)
Yellow perch (<u>Perca flavescens</u>)	0.900 (0.723-1.12)	0.0184 (0.0148-0.0229)
Channel catfish (<u>Ictalurus punctatus</u>)	0.615 (0.542-0.697)	0.0125 (0.0111-0.0142)

^a 98:2 is a mixture of TFM and Bayer 73 in the proportion 98:2 by weight based on active ingredient.

holds for the mixture i.e. the bluegill and channel catfish were the most and least resistant respectively. Other species, including salmonids and yellow perch, displayed intermediate sensitivity, although they were all considerably less resistant than the bluegill. Based on the additive index concept of Marking and Dawson (1975) (see Section 11.4), the toxicity of the mixture was strictly additive.

11.1.2 FIELD STUDIES

Some species of teleosts are killed in stream treatments, despite the fact that sea lampreys are more susceptible to the action of TFM and the 98:2 mixture. In general, observations on fish mortalities in the field are in agreement with the laboratory results with respect to sensitive species. For example, among the most susceptible large fish are the bullheads Ictalurus spp., channel catfish, white sucker and longnose sucker (Catostomus catostomus), followed by northern pike, walleye, brown trout, rainbow trout and coho salmon (U.S. Bureau Commercial Fisheries 1958, 1959, 1969; Dahl and McDonald 1980).

Minor kills of fish occur regularly, especially just below the application sites of TFM. Species most often affected include the stonecat (Noturus flavus), common shiner (Notropis cornutus), johnny darter (Etheostoma nigrum), longnose dace (Rhinichthys cataractae), blacknose dace (Rhinichthys atratulus), spottail shiner (Notropis hudsonius) brook stickleback (Culaea inconstans) and sculpin (Cottus spp.) (Dahl and McDonald 1980). Smith et al. (1974) reported that trout-perch (Percopsis omiscomaycus), logperch (Percina caprodes) and mudminnow (Umbra limi) were also affected.

Although no long-term studies have been carried out on the effects of lampricide treatments on fish populations, data from treatments and subsequent surveys have not indicated any serious depletion of indigenous species, with the exception of the stonecat (Dahl and McDonald 1980). In Lake Superior, its distribution is limited to nine streams at the extreme western end, five of which have been treated on a regular basis (Dahl and McDonald 1980). As a result, the population has been severely reduced, but not eradicated. Similarly, in Lake Ontario, where stonecats occur only in tributaries of the northwest corner, two stream treatments on Oakville and Lynde Creeks produced near complete kills (Dahl and McDonald 1980).

11.1.3 FACTORS AFFECTING THE TOXICITY OF TFM AND THE 98:2 MIXTURE

11.1.3.1 Size

Marking et al. (1975) tested different sizes of juvenile fish and found that small fish were more susceptible than large ones. For example, in hard water (300 mg.L⁻¹ CaCO₃) coho salmon weighing an

average of 1.3 g had a 24-h LC_{50} of 5.53 $mg.L^{-1}$ TFM, whereas for fish having an average weight of 7.4 g, the 24-h LC_{50} was 13.10 $mg.L^{-1}$ TFM. A similar trend was observed for rainbow trout, although the effect was less pronounced.

11.1.3.2 Spawning Condition

Fish in spawning condition are more vulnerable to the effects of TFM and the 98:2 mixture than are juveniles of the same species. This is true of dace and logperch, as well as of other species, including some salmonids (Dahl and McDonald 1980). For instance, although no serious mortality of juvenile fish was reported, brown trout in spawning condition have been killed during stream treatments (U.S. Bureau Commercial Fisheries 1961, 1968; Smith *et al.* 1974). Spawners of other species that were reported to have suffered significant mortality during TFM applications include redhorse spp., white suckers, longnose suckers and northern pike (Smith *et al.* 1974). Consequently, lampricide treatments are generally scheduled to avoid major spawning runs (Dahl and McDonald 1980).

11.1.4 TOXICITY TO EARLY LIFE STAGES

Studies on the early life stages of fish have examined the toxicity of TFM to green (newly-fertilized) eggs, eyed eggs, sac fry and swim-up fry. In general, the eyed egg stage has been shown to be the most resistant and the green egg stage the least tolerant (Olson and Marking 1973; Bills and Marking 1976; Niblett and McKeown 1980; NFRL 1983d). A recent study (NFRL 1983d), based on a 12-h exposure of different life stages of the walleye to TFM, has shown that all stages from gametes to sac fry are more resistant to TFM than are sea lamprey larvae (Table 11-3). In addition, the fertilization process was unaffected by concentrations of TFM up to 2.5 $mg.L^{-1}$, a value approaching twice the 12-h LC_{50} for ammocoetes (1.46 $mg.L^{-1}$).

Nevertheless, there is some discrepancy concerning the possibility of adverse delayed effects after exposure of the embryonic stages of fish to TFM. Niblett and McKeown (1980) observed that exposure to TFM in hard water (354 $mg.L^{-1}$ $CaCO_3$) often resulted in a delayed response in rainbow trout. For instance, during the 24-h exposure of green eggs fertilized 6 h earlier, the mortality was negligible. Yet 7-10 days later, coincident with the onset of neurulation, all eggs exposed to 2.86 $mg.L^{-1}$ TFM died. Delayed mortality, coincident with the time of hatching, and delayed hatching were observed for 21-day old eggs exposed to 5.71 $mg.L^{-1}$ as well. In addition, premature, incompletely hatched embryos occurred in eggs exposed to TFM 10-11, 21 and 32 days after fertilization. Great sensitivity to TFM seems to occur in life stages when energy demands are high (neurulation and hatching) and may be a reflection of the role of

Table 11-3. Toxicity of formulated TFM^a to eggs and other early life stages of walleye relative to sea lamprey larvae (temperature = 12°C, hardness = 44 mg.L⁻¹ CaCO₃, pH = 7.5) (from NFRL 1983d).

Life stage	Walleye 12-h LC ₂₅ (mg.L ⁻¹)	Sea lamprey larvae 12-h LC ₉₉ (mg.L ⁻¹)
Gametes	3.30	1.46
Fertilized eggs (water hardening)	2.10	1.46
12-h green eggs	1.89	1.46
Eyed eggs	5.70	1.46
Sac fry	4.70	1.46
Swim-up fry	-	-

^a TFM is a 35.7% by weight active ingredient formulation.

TFM in uncoupling oxidative phosphorylation (see Section 6.0). In contrast, the results of Olson and Marking (1973) and NFRL (1983d) showed that TFM did not produce delayed effects in either green or eyed-stage eggs of rainbow trout and walleyes, respectively.

Using a different approach, Dwyer *et al.* (1978) exposed two groups of sexually mature brook trout to concentrations of 5.71 and 6.43 mg.L⁻¹ TFM over a 12-h period to simulate a stream treatment, after which the fish were artificially spawned. No adverse effects were noted with respect to egg viability or hatchability. In addition, after 90 days, there were no differences in the survival or growth of fry in either concentration, relative to the controls.

The toxicity of the 98:2 mixture based on a 96-h LC₅₀ test using 4 early life stages of coho salmon was similar to that of TFM (Bills and Marking 1976). The eyed egg stage was the most resistant (2.64 mg.L⁻¹), followed by that of swim-up fry (1.93 mg.L⁻¹), sac fry (1.44 mg.L⁻¹) and green eggs (0.88 mg.L⁻¹). In all cases the toxicity of the mixture was additive.

11.2 SUBLETHAL EFFECTS OF TFM

11.2.1 EFFECT ON BEHAVIOR

During treatment of a model stream with 9 mg.L⁻¹ TFM over a 24-h period, Maki (1980) observed that young of the year brown trout became visibly affected. After 8 h of exposure the fish moved from their hidden, protective habitats in the shadows of the pools to unprotected, open areas. In addition, the fish became lethargic and did not respond to movements of the observer. The morning after the treatment had ceased, their behavior appeared to have returned to normal. The implication of this behavioral alteration is unclear, although it could make these fish increasingly susceptible to predation.

11.2.2 EFFECT ON GROWTH

Dwyer *et al.* (1978) exposed 2-year-old brook trout to 5.71 and 6.43 mg.L⁻¹ TFM for 12 h to simulate field conditions. No significant difference in length was found between the controls and exposed groups 100 days after the treatment. Similarly, the growth of fry from these exposed parent fish was not statistically different from that of the controls at 90 days postexposure.

11.3 ACUTE TOXICITY OF BAYER 73

11.3.1 LABORATORY STUDIES

The acute toxicity of Bayer 73 to nontarget fish is given in Table 11-4. Bayer 73 is much more toxic to all species of fish tested

Table 11-4. Toxicity of Bayer 73^a to fingerling fish (0.6-3.0 g) (temperature = 12°C, hardness = 40-48 mg.L⁻¹, pH 7.2-7.6) (from Marking and Hogan 1967).

Species	LC ₅₀ and 95% confidence interval (mg.L ⁻¹)			
	1 h	24 h	96 h	96 h ^B
Carp (<u>Cyprinus carpio</u>)	0.300 (0.267-0.338)	0.143 (0.133-0.154)	0.139 (0.134-0.145)	-
Bluegill (<u>Lepomis macrochirus</u>)	- -	0.105 (0.095-0.116)	0.094 (0.083-0.107)	0.152 (0.135-0.172)
Black bullhead (<u>Ictalurus melas</u>)	0.275 (0.230-0.329)	0.104 (0.084-0.123)	0.088 (0.078-0.098)	-
White sucker (<u>Catatomus commersoni</u>)	0.180 (0.177-0.183)	0.084 (0.073-0.097)	0.081 (0.076-0.086)	-
Yellow perch (<u>Perca flavescens</u>)	0.120 (0.103-0.140)	0.082 (0.076-0.088)	0.081 (0.069-0.087)	0.0639 (0.0568-0.0726)
Brook trout (<u>Salvelinus fontinalis</u>)	0.077 (0.069-0.086)	0.061 (0.058-0.065)	0.061 (0.058-0.065)	0.0470 (0.0364-0.0607)
Rainbow trout (<u>Salmo gairdneri</u>)	0.063 (0.060-0.066)	0.052 (0.049-0.056)	0.052 (0.047-0.054)	0.0346 (0.0297-0.0404)
Lake trout (<u>Salvelinus namaycush</u>)	- -	-	-	0.0490 (0.0433-0.0555)
Channel catfish (<u>Ictalurus punctatus</u>)	- -	-	-	0.0370 (0.0298-0.0459)
Brown trout (<u>Salmo trutta</u>)	- -	-	-	0.0282 (0.0219-0.0363)

^a Bayer 73 is a 99% by weight active ingredient formulation.

^B From Bills and Marking (1976) using 0.5-1.5 g fish.

than TFM or the mixture, with 24-h LC_{50} values $< 0.2 \text{ mg.L}^{-1}$. The 1-h LC_{50} was < 2.6 times the 24-h LC_{50} in all cases, indicating that fish responded quickly to the toxicant. Furthermore, there was no significant difference between 24- and 96-h LC_{50} s for any species listed. Based on 24-h LC_{50} s, carp (Cyprinus carpio) were about three times more resistant than the rainbow trout, the most susceptible species. Species with intermediate sensitivity, e.g. yellow perch and white sucker, were only slightly more resistant than the most susceptible ones. The 96-h LC_{50} data of Bills and Marking (1976) are in close agreement with those of Marking and Hogan (1967), with the exception of the bluegill. The former authors found the bluegill to be somewhat more resistant.

11.3.2 FIELD STUDIES

Since granular Bayer 73 is a bottom toxicant usually applied to limited areas of slow-moving water, most fish are able to escape its toxic effects, and consequently, large fish kills are rare. Problems arise when large estuarine areas are treated and the fish cannot escape. For example, after treatment of the Gawas Creek estuary of Lake Huron in 1978, treatment crews noted that hundreds of fish, including suckers spp., northern pike, bullheads spp., yellow perch and various minnow species had died (Dahl and McDonald 1980). Manion (1969) found that the treatment of Saux Head Lake (through which the Big Garlic River empties into Lake Superior) resulted in mortality to small bottom-dwelling fish such as mottled sculpin (Cottus bairdi), logperch and black bullheads (Ictalurus melas). Hence, a number of species susceptible to TFM are also sensitive to Bayer 73.

11.3.3 TOXICITY TO EARLY LIFE STAGES

Olson and Marking (1975) found that the green eggs of salmonids were resistant to Bayer 73. For example, in water with a hardness of $44 \text{ mg.L}^{-1} \text{ CaCO}_3$ and temperature of 12°C , the 24-h LC_{50} values (mg.L^{-1}) for coho salmon, chinook salmon and lake trout were > 4.25 , > 2.55 and 0.88 respectively, indicating greater resistance than fingerling fish (Table 11-4). Based on 96-h acute studies, Bills and Marking (1976) established the resistance of various stages in coho salmon, in decreasing order (mg.L^{-1}): eyed eggs (0.509), green eggs (0.286), swim-up fry (0.068) and sac fry (0.066).

11.4 MULTIPLE TOXICITY

The toxicity of separate mixtures of TFM and Bayer 73 with ammonia, carbaryl, cyanide, Delnav, DDT, endrin, malathion, nitrite, tannic acid, toxaphene, cadmium, copper, zinc and chlorine was evaluated on three species of fish: rainbow trout, white suckers and fathead

minnows in acute 96-h static tests (NFRL 1980, 1982b, 1983e; Marking and Bills 1984). Exposures of individual chemicals and paired mixtures were done simultaneously to reduce error and minimize biological variation.

Data were analyzed for LC_{50} s, 95% confidence intervals, additive indices and their ranges according to the procedure of Marking and Dawson (1975). Additive indices quantitate the combined activity of the mixture, and confidence intervals define significance. An index of zero additive toxicity indicates that the presence of additional chemicals does not cause a greater toxic effect than that which would have been expected from the toxic contributions of the individual components. Index values which are greater than zero indicate a synergistic effect, values less than zero indicate an antagonistic effect. If the confidence interval overlaps zero, the effects are considered to be simply additive.

None of the combinations produced greater than additive toxicity (Tables 11-5 and 11-6). The majority of combinations produced additive toxicity, and a few produced less than additive toxicity. Combinations of TFM with carbaryl, DDT, nitrite, toxaphene, cadmium, copper and chlorine yielded results that were less than additive for one or more of the three species. For Bayer 73, in the case of at least one of the three species, the results were less than additive for ammonia, carbaryl, endrin, nitrite, tannic acid, toxaphene, and chlorine.

The addition of more chemicals usually increases the total toxic burden with which fish must cope. If the burden of toxic units already present is high, the addition of another compound may create a lethal situation, even though its individual contribution might be sublethal. For example, lethal concentrations of nitrite nitrogen for sensitive fish species routinely found in TFM-treated tributaries, range from approximately 1 mg.L⁻¹ for rainbow trout to 6 mg.L⁻¹ for white suckers (in water with a hardness of 44 mg.L⁻¹ CaCO₃, pH = 7.5 and temperature = 12°C) (Marking and Bills 1976, 1979b). While nitrite-nitrogen concentrations of this magnitude are uncommon in natural waters, a TFM treatment for lamprey control can readily increase the toxicity of both chemicals more than twofold (Marking and Bills 1979a). Field crews can expect some mortality of sensitive fish species from the application of TFM if nitrite-nitrogen levels exceed 0.25 mg.L⁻¹. In addition, it has been suggested that the presence of pesticides, other than lampricides, may have been responsible for unexpected increases in toxicity to nontarget organisms, as sometimes occurs in the field (Meyer and Schnick 1980). As an example, carbaryl (1-naphthyl-N-methylcarbamate) added to the toxic burden of fish and reduced the amount of Bayer 73 needed to produce a given toxic effect in rainbow trout (Statham and Lech 1976).

Table 11-5. Toxicity of mixtures of TFM and selected contaminants to fish (temperature = 12°C, hardness = 44 mg.L⁻¹ CaCO₃, pH = 7.4) (NFRL 1983e; Marking and Bills 1984).

Chemical Combination	Additive toxicity index and range for		
	Rainbow trout (<i>Salmo gairdneri</i>)	White sucker (<i>Catostomus commersoni</i>)	Fathead minnow (<i>Pimephales promelas</i>)
TFM and ammonium	(-)0.14 (-)0.49 to (+)0.15	(-)0.19 (-)0.84 to (+)0.29	(-)0.06 (-)0.48 to (+)0.70
TFM and carbaryl	(-)0.37 (-)1.59 to (+)0.38	(-)0.59 (-)1.14 to (-)0.17	(+)0.53 (-)0.09 to (+)1.56
TFM and chlordane	(-)0.50 (-)1.27 to (+)0.08	-	-
TFM and cyanide	(-)0.27 -	(-)0.62 (-)1.8 to (+)0.07	(-)0.19 (-)0.74 to (+)0.23
TFM and Delnav	(-)0.26 (-)0.63 to (+)0.04	-	(+)0.32 (-)0.10 to (+)0.90
TFM and DDT	(-)0.37 (-)0.89 to (-)0.002	(+)0.05 (-)0.56 to (+)0.70	(+)1.04 -
TFM and endrin	(-)0.52 (-)1.66 to (+)0.10	(-)0.56 (-)1.55 to (+)0.04	(+)0.20 (-)0.26 to (+)0.80
TFM and malathion	(+)0.05 (-)0.28 to (+)0.42	(-)0.05 (-)0.67 to (+)0.50	(+)0.33 (-)0.11 to (+)0.95
TFM and nitrite	(-)1.25 (-)2.00 to (-)0.69	(-)0.41 (-)1.78 to (+)0.36	(+)0.36 (-)0.75 to (+)2.22
TFM and tannic acid	(-)0.43 -	(-)0.51 (-)1.47 to (+)0.08	(-)0.30 (-)0.89 to (+)0.12
TFM and toxaphene	(-)0.41 (-)0.98 to (-)0.01	(-)0.48 (-)1.19 to (-)0.001	(-)0.003 (-)0.59 to (+)0.58
TFM and cadmium	(-)0.23 (-)1.19 to (+)0.46	(+)0.07 (-)0.92 to (+)1.20	(-)0.91 (-)1.86 to (-)0.28
TFM and copper	(+)0.04 (-)0.66 to (+)0.77	(-)0.01 (-)0.84 to (+)0.79	(-)0.58 (-)1.45 to (-)0.01
TFM and zinc	(-)0.06 (-)0.72 to (+)0.52	(-)0.26 (-)1.43 to (+)0.53	(-)0.16 (-)0.88 to (+)0.39
TFM and chlorine	(-)1.04 (-)1.90 to (-)0.45	(-)0.28 (-)0.95 to (+)0.18	(-)0.63 (-)1.41 to (-)0.10

Table 11-6. Toxicity of mixtures of Bayer 73 and selected contaminants to fish (temperature = 12°C, hardness = 44 mg.L⁻¹ CaCO₃, pH = 7.4) (from NFRL 1983e; Marking and Bills 1984).

Chemical Combination	Additive toxicity index and range for		
	Rainbow trout (<u>Salmo gairdneri</u>)	White sucker (<u>Catostomus commersoni</u>)	Fathead minnow (<u>Pimephales promelas</u>)
Bayer 73 and ammonium	(-)0.22 (-)0.33 to (+)0.97	(-)0.62 (-)1.43 to (-)0.09	(-)0.11 (-)0.26 to (+)0.36
Bayer 73 and carbaryl	(+)0.34 (-)0.19 to (+)1.12	(-)0.52 (-)1.27 to (-)0.01	(-)0.07 (-)0.64 to (+)0.41
Bayer 73 and chlordane	(-)0.36 (-)1.12 to (+)0.13	-	-
Bayer 73 and cyanide	(-)0.15 (-)0.68 to (+)0.26	(-)0.21 (-)0.74 to (+)0.17	(-)0.02 (-)0.50 to (+)0.46
Bayer 73 and Delnav	(+)0.08 (-)0.72 to (+)1.01	-	(-)0.25 (-)1.00 to (+)0.29
Bayer 73 and DDT	(-)0.22 (-)1.28 to (+)0.54	(+)0.11 (-)0.45 to (+)0.74	(+)0.41 -
Bayer 73 and endrin	(-)0.88 (-)2.5 to (-)0.009	(-)0.26 (-)1.38 to (+)0.48	(-)0.38 (-)0.98 to (+)0.04
Bayer 73 and malathion	(+)0.81 (+)0.21 to (+)1.69	(-)0.03 (-)0.57 to (+)0.45	(-)0.09 (-)0.42 to (+)0.68
Bayer 73 and nitrite	(-)0.10 (-)0.72 to (-)0.42	(-)0.04 (-)0.99 to (+)0.78	(+)0.13 (-)0.77 to (+)1.24
Bayer 73 and tannic acid	(-)0.43 -	(-)0.07 (-)1.03 to (+)0.06	(-)0.43 (-)0.97 to (-)0.03
Bayer 73 and toxaphene	(+)0.03 (-)0.46 to (+)0.56	(-)0.71 (-)1.78 to (-)0.06	(-)0.37 (-)1.19 to (+)0.17
Bayer 73 and cadmium	(-)0.73 (-)2.54 to (+)0.18	(-)0.10 (-)0.91 to (+)0.59	(-)0.38 (-)1.09 to (+)0.09
Bayer 73 and copper	(-)0.22 (-)1.55 to (+)0.70	(-)0.17 (-)0.96 to (+)0.42	(-)0.16 (-)0.78 to (+)0.31
Bayer 73 and zinc	(+)0.00 (-)0.68 to (+)0.70	(-)0.42 (-)0.56 to (+)2.13	(-)0.42 (-)1.04 to (+)0.01
Bayer 73 and chlorine	(-)0.91 (-)1.96 to (-)0.24	(+)0.39 (-)0.12 to (+)1.15	(-)0.38 (-)1.05 to (-)0.08

12.0

EFFECTS OF TFM ON AMPHIBIANS AND REPTILES

12.1 EFFECTS OF TFM ON AMPHIBIANS

Information regarding the toxicity of TFM to amphibians is based on studies by Chandler and Marking (1975) and Kane *et al.* (1985), as well as on observations made in the field during treatments. No data are available concerning the effects of the 98:2 mixture or Bayer 73 alone.

Chandler and Marking (1975) determined the toxicity of TFM to three species of frog larvae (hardness = 44 mg.L⁻¹, temperature = 17°C and pH = 6.8-7.0). The 96-h LC₅₀ values for larvae of the gray tree frog (Hyla versicolor), leopard frog (Rana pipiens) and bullfrog (Rana catesbeiana) were found to be 0.78, 1.09 and 1.40 mg.L⁻¹ respectively. Using water with similar chemical characteristics (hardness = 45 mg.L⁻¹ CaCO₃, temperature = 22°C, and pH = 7.4), Kane *et al.* (1985) confirmed the sensitivity of bullfrog larvae to TFM; the 96-h LC₅₀ was 1.39 mg.L⁻¹. For free-swimming sea lamprey larvae under the same test conditions as those listed for Chandler and Marking (1975), the 96-h LC₅₀ was 0.57 mg.L⁻¹ (Dawson *et al.* 1975), indicating that all three species are more resistant to TFM than are sea lampreys. However, some mortality of gray tree frog larvae could be expected since they are only marginally more resistant than the sea lamprey ammocoetes. Indeed, 16% of treatment summaries have mentioned mortalities among frog larvae (Gilderhus and Johnson 1980). In addition, approximately 33% of field observations from Lakes Superior and Michigan listed mortalities of mudpuppies (mostly Necturus sp.) (Gilderhus and Johnson 1980). This is not surprising, considering that Howell (1966) reported the mudpuppy (Necturus maculosus) to be as sensitive to TFM as the sea lamprey. Gilderhus and Johnson (1980) concluded that mortalities in these amphibious species would be quite common during TFM applications.

12.2 EFFECTS OF TFM ON REPTILES

Little information is available concerning the toxicity of TFM to reptiles, while no published studies are available on the toxicity of the 98:2 mixture or Bayer 73 alone.

Schnick (1972) cited a study by Scherer *et al.* (1960) in which small numbers of turtles were found to be resistant to 3, 5 and 10 mg.L⁻¹ TFM at 7.7, 7.2 and 6.4°C, respectively. Similarly, Applegate *et al.* (1961) reported that 2 species of turtles, Chrysemys picta and Chelydra serpentina, suffered no mortality when exposed to concentrations of TFM ranging from 3 to 10 mg.L⁻¹ at 6.4 to 7.7°C in raceways under simulated stream conditions. In addition, reptiles were not observed to have been killed during applications of TFM (Schnick 1972).



13.0

TOXICITY OF LAMPRICIDES TO BIRDS

During the initial trial period of testing lampricides, Moffett (1958) noted that after a successful larvicide application, sea lamprey ammocoetes died by the tens of thousands and that "sea gulls at the mouth of the stream were stuffing themselves on the larvae as they washed across the sand bar into the lake." Thus birds, in addition to being exposed to lampricide-contaminated water during stream treatments, may also be exposed by consuming lampricide-containing larvae.

The effects of lampricides in the diet of birds were studied by Hudson (1979) who determined the acute oral toxicity of TFM, Bayer 73 and a 98:2 mixture to mallard ducks (Anas platyrhynchos), ring-billed gulls (Larus delawarensis), bobwhites (Colinus virginianus) and California quail (Lophortyx californicus). The acute median lethal dosages of the various lampricide formulations are listed in Table 13-1. Only mallard ducks were subjected to all four lampricides. The formulated grade TFM was more toxic ($LD_{50} = 308 \text{ mg.kg}^{-1}$) to mallards than the analytical grade TFM ($LD_{50} = 458 \text{ mg.kg}^{-1}$), possibly because of the action of the carrier, N,N-dimethylformamide (see Section 3.1). Although the mechanism of action is not known, it may have increased the absorption of TFM in the gastrointestinal tract (see Section 2.3). The toxicity of the 98:2 mixture appeared to be additive for mallards and ring-billed gulls.

Bayer 73 was much less toxic to mallards, ring-billed gulls and bobwhites than any of the TFM formulations (Hudson 1979). Overall, the ring-billed gull was the most sensitive species to formulated grade TFM, Bayer 73 and the 98:2 mixture (Table 13-1).

In addition, the toxicity of lampricide-contaminated water was evaluated in mallard ducks exposed separately to TFM, Bayer 73 and a 98:2 mixture for 48 h in ponds in their enclosures (Hudson 1979). Thereafter observations were made for 14 to 21 days. No mortalities were recorded for any of the treatments, although some signs of poisoning were evident. For example, mallards exposed to 15.8 mg.L^{-1} TFM appeared wobbly after seven hours and exhibited slight degrees of incoordination 22 h later. Birds in the 50 mg.L^{-1} group were affected after three hours, showing signs of lethargy and moderate to extreme degrees of incoordination. All birds recovered 24 to 48 h after the exposure period.

Similarly, Bayer 73 and the 98:2 mixture had little or no effect on mallards, even at levels much higher than would be used during an actual treatment. Based on the available information it appears that stream water containing a lampricide would not pose an acute threat to aquatic birds.

Table 13-1. Acute oral toxicity (LD_{50}) of lampricides in the diet of 4 species of birds (from Hudson 1979).

Species	TFM ^a		98:2 ^b mixture (mg.kg ⁻¹)	Bayer 73 ^y (mg.kg ⁻¹)
	analytical grade (mg.kg ⁻¹)	formulated grade (mg.kg ⁻¹)		
Mallard duck (<u>Anas platyrhynchos</u>)	458	308	472	> 2000
ring-billed gull (<u>Larus delawarensis</u>)	-	250	154	500
bobwhite (<u>Colinus virginianus</u>)	-	-	435	> 2000
California quail (<u>Lophortyx californicus</u>)	-	546	-	-

^a TFM is either 296% pure (analytical grade) or 35% by weight active ingredient formulation (formulated grade).

^b 98:2 is a mixture of TFM and Bayer 73 in a 98:2 ratio based on active ingredient.

^y Bayer 73 is a 70% by weight active ingredient formulation.

EFFECTS ON MAMMALS

Studies concerning the toxicity of TFM, the 98:2 mixture and Bayer 73 to mammals were conducted according to protocols designed to meet requirements of the U.S. Environmental Protection Agency for pesticide registration. Such protocols address specific questions and, as such, are unlike traditional scientific studies. Completion reports are prepared in EPA-specified formats and frequently may not be developed into manuscript formats of refereed journals. Most of the studies and reports on mammalian safety concerns fall into this category. These reports have undergone technical evaluation by scientists in the regulatory agency. The Scientific Panel considers the information in such reports, although not meeting NRCC standards for reference citations, to be useful in evaluating the available information concerning the effects of contaminants on receptors. Where possible, the Scientific Panel has extracted significant scientific information on the effects of lampricides on mammals. However, the reader must keep in mind that much of the available information contained therein has not been subjected to peer review for the primary literature. A list of completed studies is provided in Appendix III.

14.1 TOXICITY OF TFM AND THE 98:2 MIXTURE TO MAMMALS

The principal findings of the Panel developed on the basis of studies completed on the toxicity of TFM and the 98:2 mixture to mammals are the following:

1. The oral 2-week LD_{50} in adult male Sprague Dawley rats weighing 200 g was determined to be $1101 \pm 107 \text{ mg.kg}^{-1}$ TFM (active ingredient based on a 30% formulation) (WARF 1962a).
2. In a 90-day feeding study, albino rats were fed 0, 500, 900, 1620, 2916 and 5248 mg.kg^{-1} active ingredient of an 82.4% formulation of TFM daily. Body weight, feed consumption, feed efficiency, and clinical data for all groups, males and females, were within normal ranges for the age and strain of the animal used in this study. The 5248 mg.kg^{-1} ration was considered a satisfactory maximum tolerated dose (MTD) for use in high level chronic studies (WARF Inst. 1971a).
3. In an additional 90-day study, rats were fed 0, 500, 900, 1620, 2916 and 5248 mg.kg^{-1} active ingredient of a 90% formulation of TFM. It was established that TFM had a minimal effect at the levels administered. Furthermore, the level of 5248 mg.kg^{-1} was confirmed as an acceptable MTD (WARF Inst. 1971b).
4. In a 90-day feeding study, Syrian hamsters fed 0, 500, 900, 1620, 2916 and 5248 mg.kg^{-1} active ingredient of an 82.4% formulation of TFM displayed no adverse effects with respect to body weight, feed consumption or the clinical parameters measured (e.g. blood chemistry,

urinalysis data). The 5248 mg.kg⁻¹ TFM dosage level was considered a satisfactory MTD (WARF Inst. 1971c).

5. In a subacute dermal toxicity study, TFM was applied to the abraded skin of New Zealand white rabbits at dosages of 0, 150 and 300 mg.kg⁻¹ body weight for 8 h.day⁻¹, 5 days.week⁻¹, over a 3-week period. Pathological observations did not reveal any gross or histologic alterations that could be attributed to the application of TFM, other than a slight thickening of the skin at the site of application (WARF 1965).

6. To evaluate the potential effects of TFM and the 98:2 mixture on reproduction, three generations of rats were each fed at levels of 0, 300, 1250 and 5000 mg.kg⁻¹ active ingredient of an 85.6% formulation of TFM. In addition, Bayer 73 (70% formulation) was administered in two combinations with TFM so that the resulting mixtures were in a 98:2 proportion, e.g. 1250 mg.kg⁻¹ TFM + 25 mg.kg⁻¹ Bayer 73 and 5000 mg.kg⁻¹ TFM + 100 mg.kg⁻¹ Bayer 73. In no group was there any incidence of mortality or reproductive failure (WARF Inst. 1975d). Similarly, in Syrian hamsters fed 300, 1250 and 5000 mg.kg⁻¹ active ingredient of an 85.6% formulation of TFM, no apparent toxicological effects were observed on the overall reproductive performance of three generations (Warf Inst. 1975e). In addition, there was no evidence of carcinogenicity of TFM in either the rats or the hamsters (WARF Inst. 1975d, 1975e).

7. To evaluate the possibility of teratogenic effects of TFM, female rats were fed an 89.5% formulation on days 6 to 15 of gestation. From the results, TFM was not considered to be fetotoxic or teratogenic when given to rats at rates up to and including 125 mg.kg⁻¹ active ingredient (Hazelton Raltech 1983). Similarly, in rabbits, the MTD of an 85.6% formulation of TFM was not found to be teratogenic (WARF Inst. 1975c).

8. To address mutagenicity concerns about TFM, the Ames Test was conducted using a 90% formulation. TFM showed no mutagenic activity (WARF Inst. 1977).

14.2 TOXICITY OF BAYER 73 TO MAMMALS

The principal findings of the Panel from the available studies on Bayer 73 are the following:

1. In a 90-day chronic feeding study, rats were fed a daily diet containing 0, 300, 1250 and 5000 mg.kg⁻¹ active ingredient of Bayer 73. At the two highest levels, body weight was depressed. However, clinical data were normal for all groups and there were no pathological changes related to treatment as indicated by postmortem and histological observations (WARF Inst. 1974b).

2. In a 90-day chronic feeding study, male and female hamsters were fed 0, 300, 1250 and 5000 mg.kg⁻¹ active ingredient of Bayer 73 in their diet on a daily basis. Although a dose-related depression in body weight did occur among treated animals, clinical data based on hematology, blood chemistry and urinalysis showed no significant differences from the controls (WARF Inst. 1974a).
3. In a 180-day chronic feeding study, dogs were fed 0, 62.5, 250 and 1000 mg.kg⁻¹ active ingredient of Bayer 73 in their daily diet. Body weight, feed consumption and clinical data (hematology, blood chemistry and urinalysis) indicated that there was no difference between the test animals and the controls. This was confirmed by a postmortem examination of organ weights and a histological study (Procter *et al.* 1974). Similar results were observed by Hecht and Gloxhuber (1962).
4. To determine the potential contamination of the meat and milk in cattle that may ingest Bayer during a lampricide application, lactating dairy cows were given a dosage 10 times higher than that which would be found in 15 gallons of treated water. Bayer 73 was excreted rapidly, primarily in the feces and urine. Concentrations in milk residues were consistently less than 10 µg.kg⁻¹, as were those in fat, blood and muscle tissues 24 h after dosing. Concentrations in all edible portions (including the liver and kidneys) were less than 10 µg.kg⁻¹ after 72 h (WARF Inst. 1976a).
5. To evaluate the possibility of teratogenic effects of Bayer 73, New Zealand white rabbits received dosages of 0, 20, 60 and 180 mg.kg⁻¹ active ingredient of Bayer 73 between days 8 and 18 of pregnancy. Examination of the uteri for corpora lutea, live, dead or resorbed fetuses and implantations revealed that Bayer 73 did not affect fetal body weights nor cause malformations in the young (Dyck and Chappel 1975). In a similar study with new Zealand white rabbits, Levinsky and MacFarland (1974) found that concentrations of 140 mg.kg⁻¹ were not embryotoxic when administered on days 6 to 16 of pregnancy. In addition, dosages of 400 mg.kg⁻¹ produced no toxic effects in the parent.
6. Using the Ames Test, Bayer 73 was screened for potential mutagenic activity. Tests involving the 70% active ingredient W.P. revealed no mutagenic properties (Lemma and Ames 1975).
7. To address the possibility of carcinogenic effects of Bayer 73 (clonitralid), the National Cancer Institute (1978), in a long-term study, subjected rats and mice to incrementally increased dosages of Bayer 73 in their daily diets. Two feeding regimes were used for each species. With respect to rats, 50 males and females were fed either 9125 or 18 250 mg.kg⁻¹ Bayer 73 for 7 weeks, then 12 500 or 25 000 mg.kg⁻¹ respectively for 8 weeks and finally 15 000 or 30 000 mg.kg⁻¹ respectively for another 63 weeks. A 33-week period of observation followed.

Similarly, 50 male and female mice were fed either 190 or 380 mg.kg⁻¹ Bayer 73 for 8 weeks, followed by 225 or 450 mg.kg⁻¹ respectively for 15 weeks, and 300 or 600 mg.kg⁻¹ respectively for an additional 55 weeks. The animals were observed for another 14 weeks posttreatment.

The findings of the study showed that the incidence of tumors in the treated animals of both species was not significantly different from that of the controls, regardless of dosage level or sex.

8. With regard to human health, Bayer 73 has been reported to be nonirritating to the skin and mucous membranes (Gönnert 1962; Hecht and Gloxhuber 1962; Pesigan et al. 1967). In addition, it is relatively nontoxic (Gönnert 1962). Bayer 2353 (Bayer 73 is the 2-aminoethanol salt of Bayer 2353) is used as a taenicide in human and veterinary medicine (Jones et al. 1977).

Based on the information in these studies, mammals would be unlikely to suffer any toxic effects from either dermal or dietary exposure to lampricide-treated water.

APPENDIX I.0

SOME PHYSICAL-CHEMICAL PROPERTIES OF
TFM, RTFM AND BAYER 2353

Table I-1. pKa of TFM in a variety of nonaqueous solvents.

<u>Solvent</u>	<u>pKa</u>	<u>Reference</u>
isopropanol	9.9	Chantooni and Kolthoff (1979)
tertbutanol	12.7	"
dimethylsulfoxide	9.3	Chantooni and Kolthoff (1976)
dimethylformamide	10.38	"
acetonitrile	19.3	"

Table I-2. Ultraviolet-visible absorption maxima and molar absorptivities of TFM in various solvents^a (from Smith *et al.* 1960).

<u>Solvent</u>	<u>Absorption Maximum</u>	<u>Molar Absorptivity</u>
hydrochloric acid (pH 3)	280	1.93×10^3
sodium hydroxide (1%)	300	4.65×10^3
	395	1.31×10^4
ethanol	290	1.47×10^3

^a The molar absorptivities listed for the different wavelengths vary somewhat from those of Figure I-1, but are of the same magnitude.

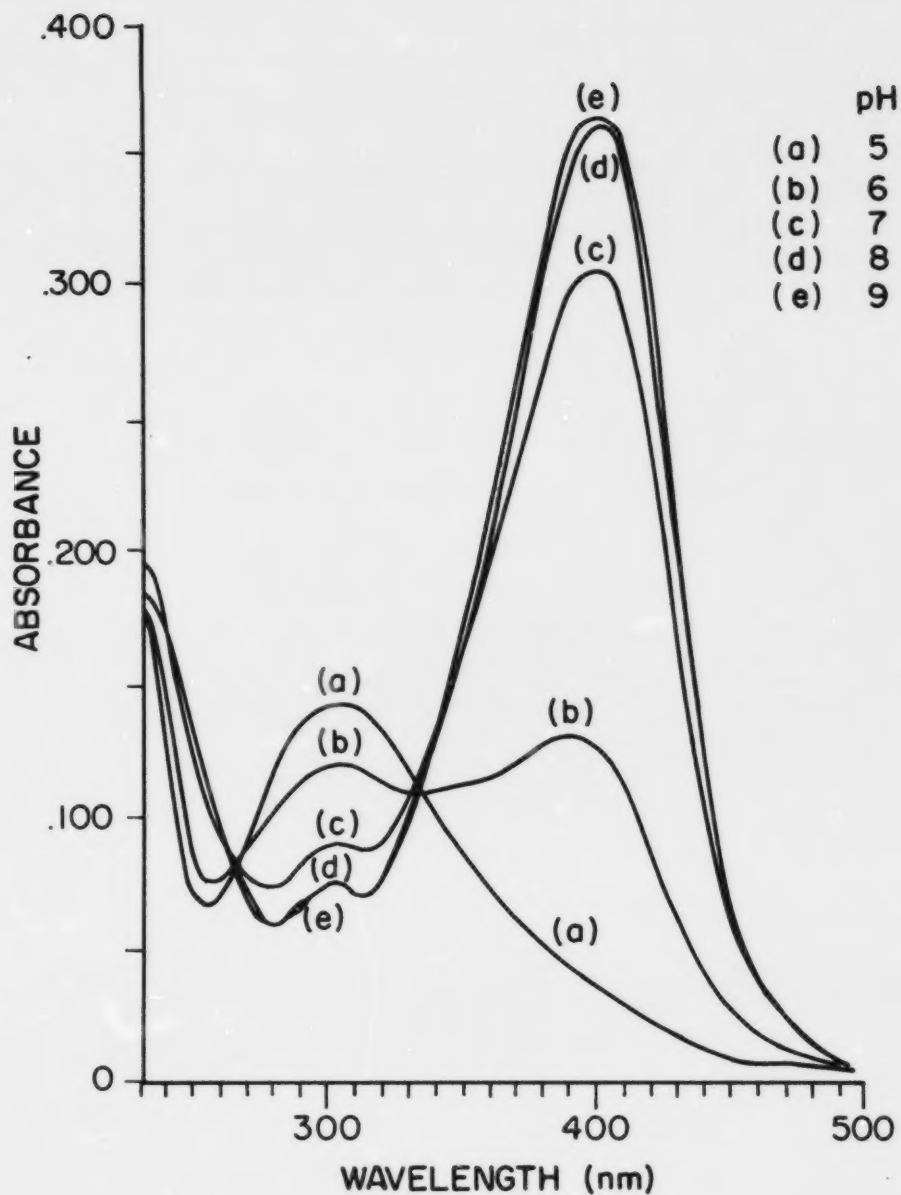
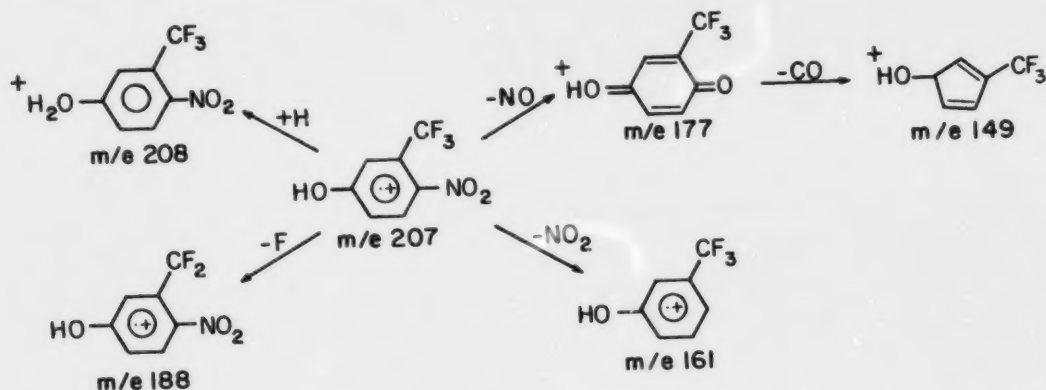


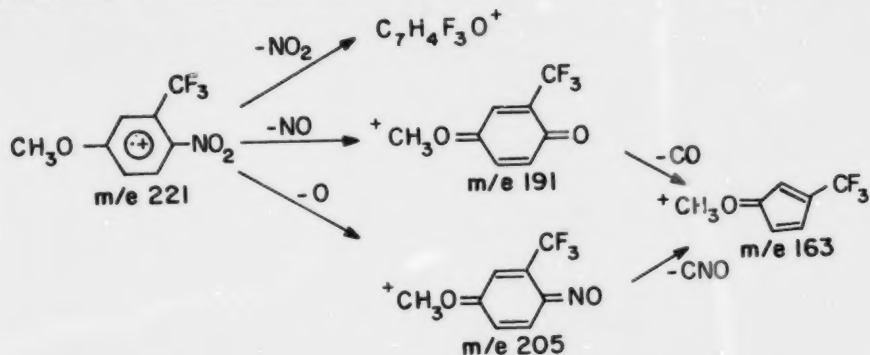
Figure I-1. Change in ultraviolet-visible absorption spectra of TFM with respect to pH (from Carey and Fox 1981).

Mass Spectra of TFM, TFM-Anisole and Bayer 2353

Mass spectra were determined using a Riber/Nermag R-1010 quadrupole mass spectrometer (Carey 1984). Samples were introduced to the mass spectrometer via a Carlo-Erba 4160 capillary gas chromatograph with a 30-m SE-52 column. The chromatograph was programmed from 90° to 250°C at 4° per minute. The methyl ether of TFM was formed using diazomethane and both the methyl ether and the free phenol were injected in ether solution using an on-column technique. Spectra were corrected for column bleed by background subtraction. The 70eV electron impact mass spectrum of TFM is shown in Figure I-2 and listed in tabular form in Table I-3. The major high mass peaks may be explained by the following fragmentation pattern:



The most common derivative of TFM used in gas chromatographic analysis is 3-trifluoromethyl-4-nitroanisole, the methyl ether of TFM. The 70 eV electron impact mass spectrum of this compound is shown in Figure I-3 and is listed in tabular form in Table I-4. The major high mass peaks may be explained by the following fragmentation pattern:



The mass spectrum of the methyl derivative of Bayer 2353 is given in Figure I-4.

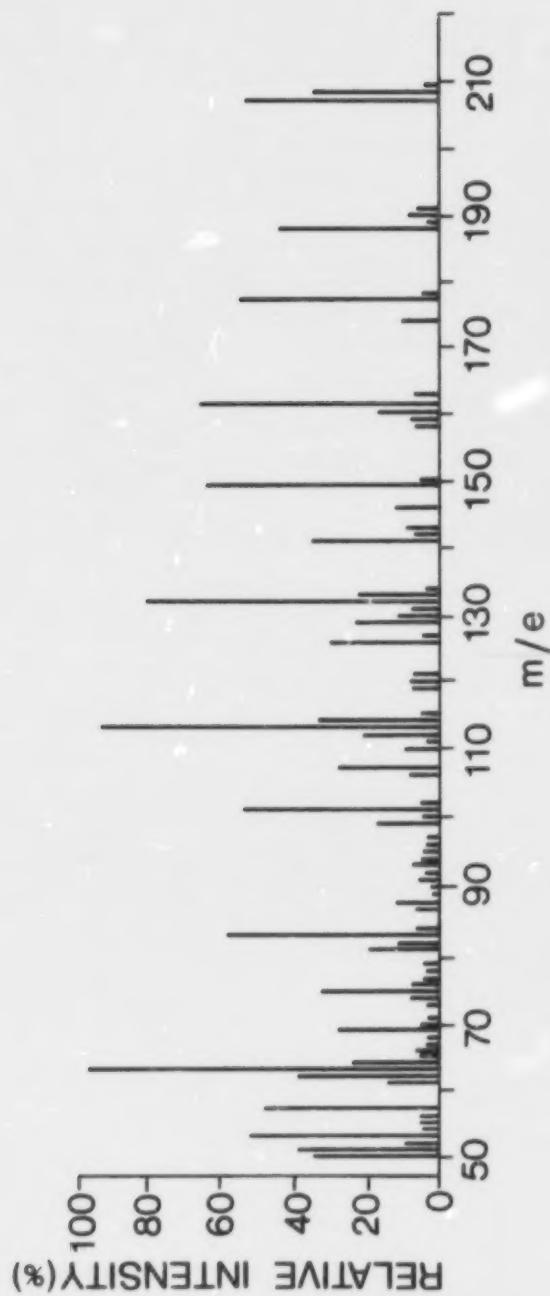


Figure I-2. Mass spectrum of 3-trifluoromethyl-4-nitrophenol (from Carey 1984).

Table I-3. Major high mass peaks in the mass spectrum of TFM (from Carey 1984).

m/e	I	m/e	I
50	35%	114	33%
51	39	126	30
53	52	129	23
57	48	132	80
62	39	133	22
63	100	141	35
64	24	149	63
69	28	161	65
75	32	162	27
83	57	177	55
101	62	188	44
107	28	207	54
112	21	208	35
113	94		

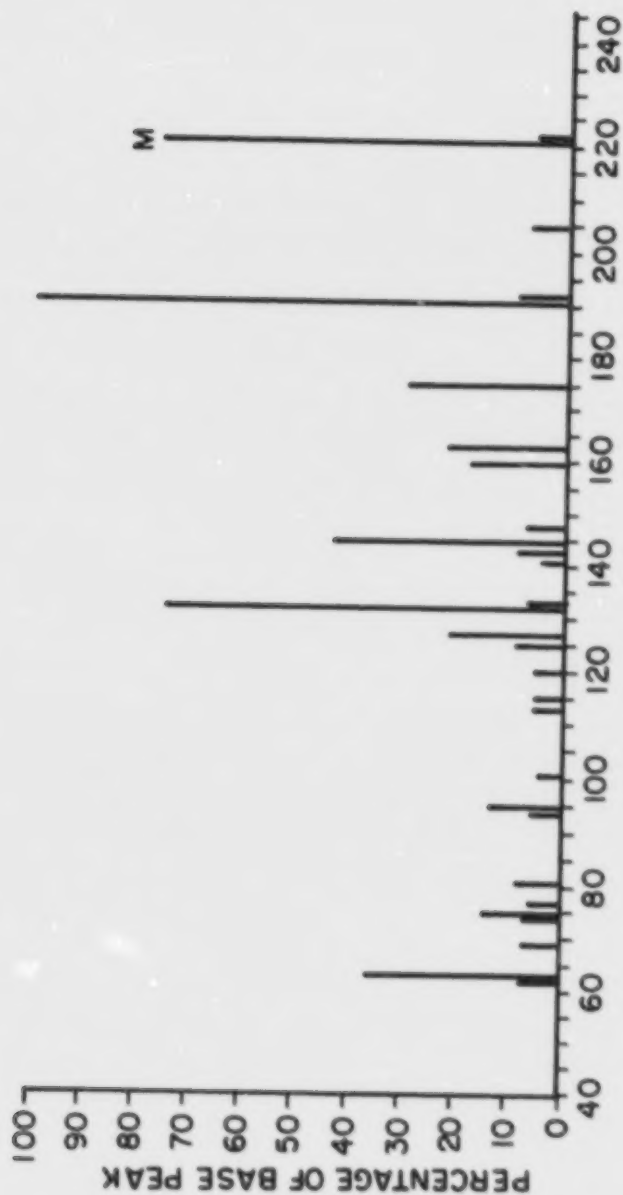


Figure I-3. Mass spectrum of 3-trifluoromethyl-4-nitroanisole (from Carey 1984).

Table I-4. Major high mass peaks in the mass spectra of TFM-anisole (from Carey 1984).

m/e	I	m/e	I
62	85	127	22%
63	37	132	75
69	8	133	8
74	8	141	5
75	15	143	10
77	6	145	44
81	9	148	8
94	6	160	18
95	14	163	22
101	5	175	30
113	6	191	100
115	6	192	10
120	6	205	7
125	9	221	77
		222	7

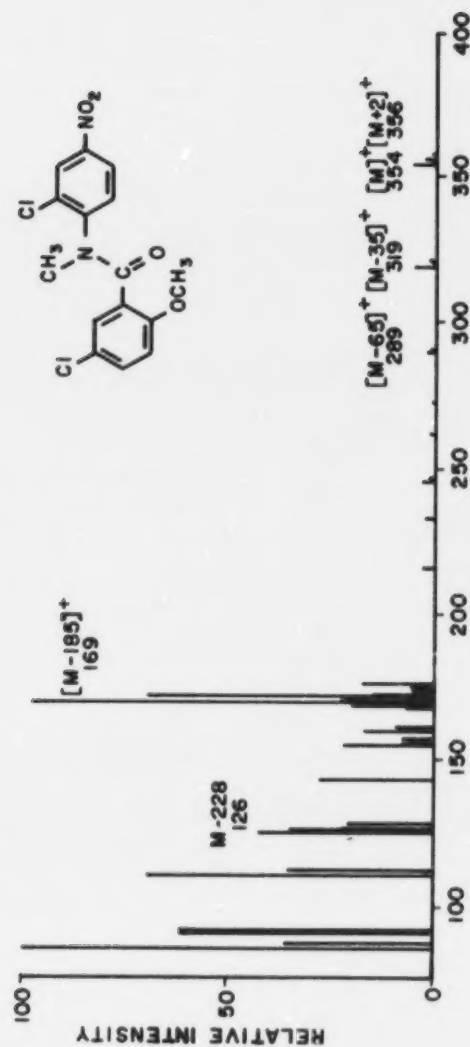


Figure I-4. Mass spectrum of methylated Bayer 2353 determined using a Finnigan 1015 mass spectrometer with direct inlet probe and 70eV ionization potential (from Muir and Grift 1980).

APPENDIX II.0

MATERIAL SAFETY DATA SHEETS ON
TFM AND BAYER 73

TFM



AMERICAN HOECHST CORPORATION

MATERIAL SAFETY DATA SHEET

DATE OF PREPARATION
November 2, 1982

Section I

MANUFACTURER'S NAME American Hoechst Corporation
STREET ADDRESS Route 202-206 North
CITY, STATE, AND ZIP CODE Somerville, New Jersey 08876
EMERGENCY TELEPHONE NO. (201) 231-2630 MEDICAL EMERGENCIES (business hours)
(201) 231-2000 Evenings and weekends
TRADE NAME Lamprecid®
PRODUCT CLASS Only for sale to and application by certified applicators of the U.S. Fish and Wildlife Service, Fisheries and Oceans Canada, and Provincial and State Fish and Game employees or persons under their direct supervision.
MANUFACTURING IDENTIFICATION A water soluble Sea Lamprey pesticide.

Section II HAZARDOUS INGREDIENTS

INGREDIENT	NATURE OF HAZARD	PERCENT	TLV			LEL	UEL	LD ₅₀
			PPM	MG/M ³	MPPCF			
a,a,a,-Trifluoro-4-Nitro-m-Cresol, Sodium Salt ^{1/}		39.9%						
Inert Ingredients		60.1%						
^{1/} equivalent to 36.1% Free Cresol								

Section III-PHYSICAL DATA

BOILING POINT 155°C MELTING POINT NA
VAPOR DENSITY (AIR = 1) 2.52 PERCENT VOLATILE NA
SPECIFIC GRAVITY 1.23 EVAPORATION RATE NA

Section IV-FIRE AND EXPLOSION HAZARD DATA

DOT CATEGORY Not regulated except when transported by aircraft. FLASH POINT 58°C LEL UEL

EXTINGUISHING MEDIA
Water, Foam, or CO₂

UNUSUAL FIRE AND EXPLOSION HAZARDS Product is likely to emit Hydrogen Fluoride and nitrogen oxides.

SPECIAL FIRE FIGHTING PROCEDURES -Keep upwind. Isolate hazard area. Avoid skin and eye contact. Avoid inhalation and use water or foam to reduce fumes.
See Section VIII - Special Protection Information

Section V-HEALTH HAZARD DATA

THRESHOLD LIMIT VALUE Toxicity Acute Oral LD₅₀ (rat) 400 mg/kg
EFFECTS OF OVEREXPOSURE Acute Dermal LD₅₀ (rabbit) 200-800 mg/kg after five applications daily.
 Most likely exposure is to skin and eyes. Concentrated Lamprecid may cause skin irritations.

EMERGENCY AND FIRST AID PROCEDURES
 If swallowed, call physician immediately. Induce vomiting and repeat until vomit fluid is clear.
 In case of contact, flush skin or eyes for at least 15 minutes. Obtain medical attention.
 Also see Patty's Industrial Hygiene & Toxicology, Vol. 2A, Pages 2573 and 2579.

Section VI-REACTIVITY DATA

STABILITY ☒ STABLE ☐ UNSTABLE
INCOMPATIBILITY (Materials to avoid) NA
CONDITIONS TO AVOID NA
HAZARDOUS POLYMERIZATION ☐ MAY OCCUR ☒ WILL NOT OCCUR
CONDITIONS TO AVOID NA

Section VII-SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED
 Take up with sand, earth or other non-combustible absorbent material or dike for later disposal.
WASTE DISPOSAL METHOD
 Call U.S. Fish and Wildlife Service, Fisheries and Oceans Canada or local fish and wildlife authorities. EPA Reg. No. 6704-45

Section VIII-SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION NA
VENTILATION Handle only in a ventilated area.
PROTECTIVE GLOVES Rubber gloves.
EYE PROTECTION Goggles or face mask.
OTHER PROTECTIVE EQUIPMENT Impermeable apron, leg protection and waterproof boots.

Section IX-SPECIAL PRECAUTIONS

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORING
 Store in a cool dry storage area where contamination of fertilizer, seed, foodstuffs or other materials will not occur.
OTHER PRECAUTIONS
 Keep out of all water supplies.
 Do not get in contact with skin or clothing. Change protective clothing daily or when contaminated.

Product Safety Committee
 American Hoechst Corporation
 Route 202-206 North
 Somerville, New Jersey 08876

PRECAUTIONARY STATEMENTS HAZARDS TO HUMANS (& DOMESTIC ANIMALS)

DANGER

For use by trained operators only. May be fatal if swallowed or absorbed through skin. Avoid contact with skin and eyes. May produce severe burns. Concentrated forms of LAMPRECID: CID. Protective clothing, rubber gloves, and face masks are necessary for the minimum protection of handlers. Wash thoroughly after handling.

ENVIRONMENTAL HAZARDS

Directions for use must be strictly followed to minimize hazardous waste. Do not contaminate water by cleaning of equipment or disposal of wastes.

Local, State, and Provincial Fish and Game Agencies must be contacted before product is applied. Municipalities that use streams requiring treatment as potable water sources must be notified of the impending treatment at least 24 hours prior to application. Treatment of streams requiring irrigation water must be notified of the impending treatment at least 24 hours prior to application. Agricultural irrigators must turn off their irrigation system for a 24-hour period during and after treatment.

Not to be used by unauthorized personnel.
Nr. 203B

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling. SEE RIGHT PANEL FOR DIRECTIONS.

CATEGORY OF APPLICATOR

Aquatic pest control

STORAGE AND DISPOSAL

Do not contaminate water, food, or feed by storage or disposal. Open dumping is prohibited.

STORAGE: Containers of LAMPRECID should be stored in an area where spills or leakage of the material can be contained.

DISPOSAL: LAMPRECID spray mixture or residue that cannot be used or chemically reprocessed should be disposed of in a landfill approved for pesticides or buried in a safe place away from water supply after container is triple rinsed for recleaning, or disposal in approved landfill or bury in a safe place. Consult federal, provincial, state or local authorities for approved alternative procedures.

DIRECTIONS FOR USE

Pretreatment surveys are always made to determine abundance of sea lamprey larvae (Petromyzon marinus). Each body of water on the Great Lakes selected for treatment must first be analysed on site to determine both the minimum concentration of LAMPRECID required to kill sea lamprey larvae and the maximum concentration that can be applied without causing undue mortality of other fish species. A "leakage" consisting of a 10% margin of safety or the use of a multiple regression formula based on the results of a multiple regression analysis of the body of water must be determined. The analysis and conductivity of the body of water.

The concentration of LAMPRECID required to kill sea lamprey larvae is determined by the water chemistry and temperature. Measure volume or flow-rate and add the amount of LAMPRECID necessary at rates based on the foregoing analysis. Dispense LAMPRECID by application devices sufficiently accurate to maintain predetermined concentration. Concentration in the body of water must be determined either by colorimetric, chemical, or photometric methods, or high performance liquid chromatography.

LAMPRECID may be used by itself in the treatment of bodies of water in the Great Lakes. The minimum concentration of 70% Wettable Powder (EPA REG. No. 3125-135) may be used in conjunction with LAMPRECID (EPA REG. No. 6704-45) for control of sea lamprey larvae. Application of BAYLUSCIDE 70% Wettable Powder may be made as a simultaneous addition with LAMPRECID on larger rivers to reduce the amount of LAMPRECID required or as a subsequent addition to the stream to determine lamprey populations. When using BAYLUSCIDE 70% Wettable Powder in combination with LAMPRECID, mix in proportions so as to result in a final concentration of BAYLUSCIDE 70% Wettable Powder of not more than 2.5% for LAMPRECID by weight. BAYLUSCIDE 70% Wettable Powder may be added to LAMPRECID in two ways.

1. One method of application is to add a slurry of BAYLUSCIDE 70% Wettable Powder pumped into the stream through a pump while the LAMPRECID is being applied. Through a second method, the LAMPRECID is calculated to deliver the desired rate of application and the BAYLUSCIDE 70% Wettable Powder is easily mixed as a slurry than by direct mixing with LAMPRECID and more uniform dispenser rates result.

2. A second application method is used on the large river systems with multiple tributaries. The number of application sites on these large rivers precludes the use of the first method because of the number of feeder pumps involved, the need for a 110-volt power source at each site to run a pump, and the often difficult access to sites. On these large river systems, LAMPRECID is applied to the main stem of the river. BAYLUSCIDE is introduced into the chemical bank in amounts calculated to produce the desired LAMPRECID to BAYLUSCIDE ratio. The LAMPRECID applications in tributaries are timed so that the individual chemical banks meet and to a chemical mix in the main stream. The chemical mix is then directed by ground water, swamp seepage, untreated tributaries, occasional rain, or other conditions that cannot be included when the application rates for the tributaries are calculated. The toxicity of the bank in a main stem is increased by the addition of LAMPRECID to BAYLUSCIDE. The increased toxicity of the BAYLUSCIDE-LAMPRECID mix over LAMPRECID alone to sea lamprey larvae.

RESTRICTED USE PESTICIDE

ONLY FOR SALE TO AND APPLICATION BY CERTIFIED APPLICATORS OF THE U.S. FISH AND WILDLIFE SERVICE, FISHERIES AND OCEANS CANADA, AND PROVINCIAL AND STATE FISH AND GAME EMPLOYEES OR PERSONS UNDER THEIR DIRECT SUPERVISION.

SEA LAMPREY LARVICIDE LAMPRECID®

ACTIVE INGREDIENT:
α, α, α-TRIFLUORO-4-NITRO-m-CRESOL, SODIUM SALT: _____ %
EQUIVALENT TO _____ % FREE CRESOL

INERT INGREDIENT: _____ %

TOTAL: 100.00%
PER GALLON

THIS PRODUCT CONTAINS _____ LBS. OF _____

KEEP OUT OF REACH OF CHILDREN
DANGER-POISON



STATEMENT OF PRACTICAL TREATMENT

NO KNOWN EFFECTIVE ANTIDOTE:

IF SWALLOWED, CALL PHYSICIAN IMMEDIATELY. INDUCE VOMITING by giving milk or white of egg beaten in water, then a tablespoon of salt in glass of warm water and repeat until vomit fluid is clear. Repeat milk or white of egg beaten with water.

IF ON SKIN, flush liberally with water for at least 15 minutes and wash with soap if possible.

IF IN EYES, flush liberally with water for at least 15 minutes; get medical attention.

SEE LEFT PANEL FOR ADDITIONAL PRECAUTIONARY STATEMENTS



Hoechst

LBS

MFG. BY: HOECHST AKTIENGESELLSCHAFT
TOWN, COUNTRY: 65926 Frankfurt/Main, FR - Germany
PACKED FOR: U.S. FISH AND WILDLIFE SERVICE
STATE: WASHINGTON, D.C. 20240
ESTABLISHMENT NO. 8340-WG-4
EPA REGISTRATION NO. 6704-45

NET CONTENTS

BAYER 73



MATERIAL SAFETY DATA SHEET

MOBAY CHEMICAL CORPORATION - PITTSBURGH, PA 15205

AUG 20 1980

MANUFACTURING DIV. ADDRESS

AGRICULTURAL CHEMICALS DIVISION, P. O. Box 4913,
Kansas City, Missouri 64120

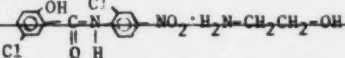
DATA SHEET NO. 5013

DIVISION Agrl. Chem.

ISSUE DATE 3-17-80

ISSUED BY CDS

SUPERSEDES None

CHEMTREC CHEMICAL TRANSPORTATION EMERGENCY TELEPHONE NO. 800-434-9200; DISTRICT OF COLUMBIA: 202-462-7818		MOBAY NON-TRANSPORTATION EMERGENCY NO. (816) 242-2582	
PRODUCT NAME BAYLUSCIDE 70 W.P.		PRODUCT CODE NUMBER 011087	
CHEMICAL FAMILY Amino ethanol salt		CHEMICAL NAME & SYNONYMS 2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide	
CHEMICAL FORMULA 		TRADE NAME & SYNONYMS BAY 25648	

HAZARDOUS INGREDIENTS

HAZARDOUS MIXTURES OF OTHER LIQUIDS, SOLIDS OR GASES	%	CURRENT TLV
Bayluscide	70	

PHYSICAL DATA

APPEARANCE (SOLID, LIQUID, GAS) Solid	MOLECULAR WEIGHT 388.1	MELT POINT NE	SPECIFIC GRAVITY NA
VAPOR DENSITY (AIR=1) NA	COLOR Yellow	BULK DENSITY NE	BOILING POINT NA
POR PRESSURE NA	SOLUBILITY NE	ODOR NE	% VOLATILE BY VOLUME NE

FIRE & EXPLOSION DATA

FLASH POINT °F (METHOD USED) NA	FLAMMABLE LIMIT Lel --- Uel ---	EXTINGUISHING MEDIA Dry chemical or water
SPECIAL FIRE FIGHTING PROCEDURES NA		
UNUSUAL FIRE OR EXPLOSION HAZARDS		

TOXICITY DATA

LD50, ORAL (INGESTION) 7500 mg/kg	LD50, DERMAL (SKIN CONTACT) > 2000 mg/kg	INHALATION LC50 < 2000 mg/L/hour
FISH, LC50 (LETHAL CONCENTRATION) Bluegill = .1 ppm (24 hrs.)	TLV (UNITS) (THRESHOLD LIMIT VALUE) NE	SKIN IRRITATION NE
EFFECTS TO EYE NE	EFFECTS TO LUNG NE	OTHER Toxic to fish & crustaceans

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
XXXXXXXXXXXXXXXXXXXXXXXXXXXX

EMERGENCY AND FIRST AID PROCEDURES, EFFECTS OF OVER EXPOSURE

If swallowed induce vomiting. Remove contaminated clothing and wash skin and hair

thoroughly with soap and water. Consult physician.

REACTIVITY DATA

STABILITY NE	CONDITIONS TO AVOID
POLYMERIZATION NE	CONDITIONS TO AVOID
INCOMPATIBILITY (MATERIALS TO AVOID)	
HAZARDOUS DECOMPOSITION PRODUCTS	

SPILL OR LEAK PROCEDURE

STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED	
Sweep up spilled material and place into container. Scrub area with soap and water. Rinse with water.	
WASTE DISPOSAL METHOD	
Bury in State or EPA approved landfill. Avoid any contamination of streams or ponds.	

SPECIAL PROTECTION DATA

RESPIRATOR TYPE	
NIOSH approved chemical cartridge respirator with pesticide cartridges and dust pads	
EYE PROTECTION	GLOVES
Safety glasses	Latex
OTHER PROTECTIVE EQUIPMENT	
Launder clothing daily after use	

SPECIAL PRECAUTIONS & STORAGE DATA

STORAGE TEMPERATURE		AVERAGE SHELF LIFE
Min.	Max.	
SPECIAL SENSITIVITY (HEAT, LIGHT, MOISTURE)		
PRECAUTIONS TO BE TAKEN IN HANDLING AND STORING		
Do not store near any materials intended for use or consumption by humans or animals.		

SHIPPING DATA

DOT SHIPPING NAME		TECHNICAL SHIPPING NAME
Insecticides, O/T Agricultural		BAYLUSCIDE 70% W.P.
DOT HAZARD CLASSIFICATION		Secondary:
Primary:		
DOT LABELS REQUIRED		FREIGHT CLASSIFICATION
NA NA		

REASON FOR ISSUE
Initial issue

INITIATED BY	TITLE	APPROVED BY	TITLE
L. R. Thompson	Industrial Hygienist	<i>[Signature]</i>	Industrial Hygiene Air Quality Manager
DATE INITIATED		DATE APPROVED	
March 6, 1980		March 7, 1980	

NE - NOT ESTABLISHED NA - NOT APPLICABLE

This information is furnished without warranty, expressed or implied, except that it is accurate to the best knowledge of Mobay Chemical Corporation. The data on this sheet relates only to the specific material designated herein. Mobay Chemical Corporation assumes no legal responsibility for use or reliance upon these data.

SUPPLEMENTAL LABELING

^(B) BAYLUSCIDE

70% WETTABLE POWDER

ACTIVE INGREDIENT:

5-Chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide
compound with 2-aminoethanol(1:1) 70%

INERT INGREDIENTS: 30%
100%

U.S. Patent Nos. 3,079,297 and 3,113,067
EPA Reg. No 3125-136

DIRECTIONS FOR USE FOR CONTROL OF SEA LAMPREY LARVAE

ONLY FOR RETAIL SALE TO AND APPLICATION BY CERTIFIED APPLICATORS OF THE U.S. FISH AND WILDLIFE SERVICE, ENVIRONMENT CANADA, AND PROVINCIAL AND STATE FISH AND GAME EMPLOYEES OR PERSONS UNDER THEIR DIRECT SUPERVISION.

State and Provincial Fish and Game Agencies must first be contacted before product is applied.

BAYLUSCIDE 70% Wettable Powder may be used as a synergist in combination with TFM (EPA Reg. No. 6704-45) for control of sea lamprey larvae (*Petromyzon marinus*) in streams and tributaries of the Great Lakes. Application of BAYLUSCIDE 70% Wettable Powder may be made as a simultaneous addition with TFM on larger rivers to reduce the amount of TFM required or as a subsequent addition downstream to enhance TFM larvicidal activity.

Prior to using BAYLUSCIDE-TFM, pretreatment surveys must be made to determine larvae populations.

Each stream selected for treatment must first be analyzed on site to determine both the minimum concentration of material required to kill lamprey larvae and the maximum concentration that can be applied without causing undue fish mortality. "Analysis" constitutes live animal bioassay or the use of a regression established by past bioassays and total alkalinity and conductivity of stream water.

Lethal concentration may vary depending upon water chemistry and temperature. Carefully calculate stream flow-rate and add the amount of material necessary to kill lamprey larvae with minimal fish mortality. Metering devices will be used that accurately deliver application rates as calculated. Chemicals will be monitored colorimetrically or by gas chromatography to insure that minimum lethal concentrations for sea lampreys are maintained and maximum allowable concentrations are not exceeded.

Directions for Mixing and Application: When using BAYLUSCIDE 70% Wettable Powder as a synergist in combination with TFM, mix in proportions so as to result in a final concentration of BAYLUSCIDE 70% Wettable Powder of not more than 2% of TFM by weight. BAYLUSCIDE 70% Wettable Powder may be added to TFM in 2 ways:

1. One method of application is a slurry of BAYLUSCIDE 70% Wettable Powder pumped into the stream through a pump while the TFM is fed separately through a conventional fuel pump feeder in amounts calculated to deliver the desired rate of TFM to BAYLUSCIDE. BAYLUSCIDE is more easily mixed as a slurry than with TFM and more uniform feed rates result.
2. A second application method is used on the large river systems with multiple tributaries. The number of application sites on these large rivers precludes the use of the first method because of the number of feeders involved, the need for a 110-volt power source at each site to run a pump, and the often difficult access to these sites. On these large systems, TFM alone is fed into the tributaries. Where the tributaries join to form the main stream of the river, BAYLUSCIDE is introduced into the chemical bank in amounts calculated to produce the desired TFM to BAYLUSCIDE ratio. The TFM applications in tributaries are timed so that the individual chemical banks meet and form a chemical bank in the main stream that approximates the chemical concentrations in the tributaries. Since the banks are diluted by ground water, swamp seepage, untreated tributaries, occasionally rain, or other conditions that cannot be included when the application rates for the tributaries are calculated, the toxicity of the bank in the main stream must be raised by the addition of TFM or BAYLUSCIDE. The latter can be used in place of TFM because of the increased toxicity of the TFM-BAYLUSCIDE mixture over TFM alone to sea lamprey larvae.



Chemagro Agricultural Division
Moba Chemical Corporation
Box 4913, Kansas City, Mo. 64120

878050

This bulletin is not intended to provide adequate information for use of this product. Before using BAYLUSCIDE 70% Wettable Powder, read and carefully observe caution statements, directions for use, restrictions and disposal information on the label. Observe all cautions and limitations on labeling of all other products used in combination with BAYLUSCIDE.

APPENDIX III.0 REFERENCES - MAMMALIAN SAFETY STUDIES

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